CHARACTERIZATION OF EAST AFRICAN ACCESSIONS OF *Musa* AAB "APPLE" AND *Musa* AA "MURARU" DESSERT BANANAS

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I dedicate this work to my husband James Onyango Ouma and children Mathew Huma, Annette Auma, Emmy Awuor and Wilfrida Achieng, and to my late brother Mark Onyango.

ABSTRACT

One of the major hindrances to future improvements in bananas and plantains production in East Africa is the endless range of names and synonyms used to describe different cultivars and the lack of understanding of their true biological relationships. The East African AAB "Sukari Ndizi" and AA "Muraru" dessert bananas are prime examples of this confusion. To better understand these two groups of bananas and to evaluate them for economically important traits, vital information such as the identity and distinctness of cultivars needed to be developed. This study had the following general objectives: (1) to use molecular and morphological tools to analyze the variation that exists within the East African AAB "Sukari Ndizi" and AA "Muraru" dessert bananas, (2) to classify AAB "Sukari Ndizi" and AA "Muraru" dessert banana cultivars into distinguishable groups for ease of reference and communication, (3) to identify their key characters for development of a provisional identification system, and (4) to identify superior AAB "Apple" and AA "Muraru" cultivars for production purposes. In this study, the classification of the East African AAB "Sukari Ndizi" and East African AA "Muraru" bananas was achieved using molecular microsatellite markers, morphological markers, flow cytometry ploidy analysis and horticultural trait evaluation. Microsatellite markers from both nuclear and chloroplast DNAs were useful for distinguishing the various bananas groups, and to separate 4 taxa of AAB "Apple" dessert banana accessions using both cluster and principal component analysis. Using cluster analysis, the "Sukari Ndizi" were classified as distinct taxon within the AAB "Apple" dessert bananas. Flow cytometry analysis also confirmed that "Sukari Ndizi" is triploid AAB and "Muraru" is a diploid

AA. Cluster analysis based on microsatellite data showed "Muraru" to be distinct taxon from other AA accessions, and very closely related to the commercial AAA dessert bananas. Morphological studies have also identified key characters exclusive to these two banana groups that can be used for development of provisional identification systems. Finally, horticultural analysis of several cultivars was carried out using various traits, and these cultivars can be recommended for further production in the region. This study demonstrated that microsatellite markers are useful and powerful tools for banana classification and for the analysis of biological relationships. Flow cytometry determined the ploidy levels of the banana accessions. Analysis of variance of replicated accession samples and the use of *Pisum sativum* as an internal standard with flow cytometry, made it possible to predict the actual genomic composition of various accessions.

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CHAPTER 1

INTRODUCTION

The name "banana" was recorded (Bakshi 1963) as originating from the Guinea coast of West Africa, possibly from the Sherbro or Temme languages of Sierra Leone in the sixteenth century (Rowe and Rosale 1996). Although bananas are natives to Papua New Guinea (PNG), they were probably transferred from Indonesia to Africa via Madagascar about the fifth century AD (Baker and Simmonds 1951, Simmonds 1962) and are now grown in more than 120 countries of the world for its fruit. In banana importing countries, where only the banana fruit is seen, "banana" usually stands for the soft, sweet dessert banana fruit eaten ripe but raw. Yet a large amount of bananas produced in the world do not fall into this category as they have firmer, starchier fruit and are generally used in cooking. Banana fruit may also be dried and ground into banana flour. There are two basic kinds of banana plants: those that produce seedless, edible fruit and those that are wild and produce seedy fruits. Although the wild bananas have fruit with numerous large, hard seeds, almost all edible bananas are seedless.

Edible bananas are subjectively classified into four groups based on their use: cooking, beer, roasting and dessert. In East Africa, i.e., Kenya, Uganda, Tanzania, Rwanda, and Burundi, cooking bananas serve as a staple food while dessert bananas are the least expensive fruit. All four types of bananas are grown in East Africa, except in Kenya where beer and roasting types are rare. Cooking and dessert bananas are prevalent in Uganda, Tanzania, and Kenya; Rwanda and Burundi have mostly

beer bananas. However, in general, the suitability of a banana type for a particular use is governed largely by cultural and consumer preferences (Damello *et al.* 2001). This is the case in East Africa where many banana types are culturally classified as dual purpose, i.e., the fruit is consumed either fresh or cooked. The selection of a particular type of banana to grow also depends on consumer preference.

Banana cultivars (cultivated varieties of bananas) are also classified by horticulturalists by their genetic composition. Cultivated banana plants are mostly diploid and polyploid hybrids of two wild banana species, *Musa acuminata* and *Musa balbisiana* (Stover and Simmonds 1987). Ploidy and genome composition of the different clones are designated by A and B to represent the genomes of *M. acuminata* and *M. balbisiana*, respectively (Rowe and Rosale 1996). Within the dessert bananas, for example there are AAA (i.e. triploid bananas composed of only *M. acuminata*), or AA (diploid bananas only of *M. acuminata*) and AAB (triploid bananas composed of 2/3 *M. acuminata* and 1/3 *M. balbisiana)*. The same genomic classification applies to cooking and beer bananas. However roasting bananas belongs to only one genomic group, AAB; these are popularly known as plantains.

East Africa (to the West and North of Lake Victoria) is regarded as a secondary centre of diversity for bananas (Baker and Simmonds 1951, Stover and Simmonds 1962). Bananas in East Africa have been used for centuries as an important food and cash crop for about 20 million people (INIBAP 1986). The annual banana production of East and Southern Africa is over 20 million tonnes (Karamura *et al.* 2000), and East Africa has all the cultivated groups of *M. acuminata* and *M. balbisiana* hybrids. The most abundant banana types are the East African

highland AAA bananas (AAA-EA). Other cultivars include the AAB plantains, the ABB types, the commercial dessert AAA bananas (Gros Michel and Cavendish), the AAB East African small fruited dessert banana referred to here as "Sukari Ndizi" and the East African cultivated diploid dessert banana referred to here as "Muraru". These two groups are very important in the region, but have not been studied. About 5% of the bananas grown in Kenya and 3% of those in Uganda belong to the AB/AAB dessert group (Karamura *et al.* 1996). The "Muraru" dominates in Central Kenya as the dessert banana and is also grown extensively in Arusha, Tanzania. The "Sukari Ndizi" and "Muraru" bananas constitute roughly 5-10 % of the total banana germplasm in East Africa and about 50% of the dessert banana varieties grown in the region (Personal observation). The "Sukari Ndizi" was found to be the most ubiquitous cultivar in Uganda (Gold *et al.* 2002). An expedition in Tanzania in 2001 also collected many non-reported diploids AA (De Langhe *et al.* 2002) that were established in Tengeru, Arusha, Tanzania.

Dessert bananas grown in East Africa are grouped into four main types: AAA Gros Michel and Cavendish, AB/AAB "Apple", AA "Muraru" and the AAA East African Highland cooking banana. Because Cavendish and Gros Michel are major export bananas for international commerce, much research has been concentrated on them. The "Apple" AAB and "Muraru" AA groups are grown for local consumption and hold a special place as dessert bananas in East Africa. They meet consumer taste preferences in many regions, and local demand for both of them is high. The "Apple" banana also has a potential export market (Karamura *et al.* 2000).

Major constraints to commercial banana production in East Africa include susceptibility to serious pests and diseases. The major diseases of banana in East Africa are panama or fusarium wilt, caused by *Fusarium oxysporum fsp.* Cubense race1 (Kungu *et al.* 2001); sigatoka leaf spot, caused by the fungal pathogens *Mycosphaerella fijiensis* and *Mycosphaerella musicola*;, and the recently identified bacterial wilt, caused by *Xanthomonas campestris* pv. *Musacearum.* Banana weevils (*Cosmopolites sordidus* (Germar)) and nematodes (*Radopholus similis*, *Helicotylenchus multicinctus, Pratylenchus goodeyi,* Meloidogyne spp) are the most important pests. For some of the pests and diseases, such as fusarium wilt and sigatoka, genotype does seem to play a role in conferring resistance, as these diseases do not affect all bananas. Identification of banana types that are tolerant or resistant to some of these diseases and pests would be a major step towards improving banana production. However, it is pointless to evaluate varieties without proper identification. The importance and utility of having proper varietal identification tools in any crop improvement program cannot be overstated. While improvements in banana production in East Africa have been made based on agronomy, especially crop husbandry, one of the major hindrances to future progress into bananas and plantains is the issue of the endless range of names and synonyms of cultivars. The "Sukari Ndizi" and "Muraru" bananas are no exception to this confusion.

Because the "Sukari Ndizi" and "Muraru" groups include such a large fraction of the dessert group of bananas found in East Africa, any advancement using them will have a potentially large economic impact in the region. The "Sukari Ndizi" and "Muraru" bananas are mostly grown in the western and central (the Gikuyu) regions

of Kenya, Zanzibar, Moshi, Bukoba, Morogoro and Usambara-Kilimanjaro axis of mountains of Northeastern Tanzania (Baker and Simmonds 1951, Rossel and Mbwana 1991, de Langhe *et al.* 2002, Karamura *et al.* 2006). "Sukari Ndizi" bananas are also found in parts of Uganda and Rwanda. They are commonly referred to as "Sukari" or "Sukari Ndiizi" in Uganda and as "Kamaramasenge" in Rwanda. Another cultivar commonly confused with "Sukari Ndizi" because of a similarity in fruit characteristics is 'Kisubi' in Uganda and "Gisubi kagongo" in Rwanda. Overall, the extent of diversity within the "Sukari Ndizi" and "Muraru" groups is not known. In order to better understand these two groups of bananas and to evaluate them for economically important traits, vital information such as the identity and distinctness of the varieties grown needs to be collected. It is also crucial to be able to distinguish between cultivars based on key characteristics, even at the nursery level, since one must be able to buy a stock of plantlets and be sure that it will show the expected characteristics.

Many of the National Agricultural Research Systems in the East African region maintain germplasm collections of local varieties and accessions that have not been fully characterized. The banana research network for East and Southern Africa (BARNESA) (1998) proposed that characterization studies be conducted on endemic germplasm with regard to taxonomic and agronomic characters to build on the morphometric characterization already carried out by Karamura (1998). Some benefits of the characterization studies include information on the value, potential, and limitations of germplasm, and the facilitation of utilization of genetic resources in plant breeding. The "Sukari Ndizi" and "Muraru" bananas in East Africa are poorly

studied and have never been fully described, although several broad studies have been conducted in the East Africa region, and these two groups of bananas are specifically mentioned in the literature (Baker and Simmonds 1948, Sebasigari 1987). In some of the collections, accessions have been identified and names compiled (Stover and Simmonds 1987, Evers 1992, Pillay *et al.* 2000, 2001). Evers (1992) collected new accessions from the Morogoro region and identified them to group and sub-group level based on morphological methods, and noted some unexpected variations in especially AAB and ABB groups. Profiling of bananas in the region in general using RAPDs has also been done (Kahangi *et al.* 2002, Onguso *et al.* 2004).

A number of clones of "Sukari Ndizi" and "Muraru" banana accessions have been described with various names. Although some within these two groups are probably just synonyms of the same accessions, as the number of named variants increases, it becomes necessary to group them in some way to indicate shared or different attributes. It is essential to confirm whether the names are describing genetically distinct individuals and to find out how much diversity there is within these groups. The following are some of the names currently applied indiscriminately to these bananas. For "Sukari Ndizi": Kanana, Sukali Ndiizi, Kabaragara, Kamaramasenge, kipukusa, Pukusa, Kipukusa cha Java, Bunana, Kasukari, Kisukari, Kisubi, Subi, Kasubi, Gisubi Kagongo, Silk, Mysore, Lady's finger, Sucrier, Kisukari Kavu, Kisukari Chachu, Kipakapaka Ndogo, Kipakapaka Kubwa, Apple, Sweet banana, Ney Poovan, and Wangae. For the "Muraru": Mshale, Mjenga, Mulalu, Muraru, Mlelembo, Makhughu, Njuru, Kamunyilya, Sucrier, Lady's finger, and Mraru.

The current use of names is confusing and the questions that need to be asked include:

1. Are the East African AAB "Sukari Ndizi" and AA "Muraru" dessert bananas distict taxon? If so, is there diversity within each of them, and what is the extent of that diversity?
2. What is the relationship of AAB "Sukari Ndizi" and AA "Muraru" dessert bananas to other cultivated bananas?
3. How does one distinguish the East African AAB "Sukari Ndizi" and AA "Muraru" bananas?
4. Are there horticulturally superior accessions within both AAB "Sukari Ndizi" and AA "Muraru" that can benefit farmers in the East African region?

Variability within the East African "Sukari Ndizi" and "Muraru" banana populations could be useful in a breeding program. Germplasm material may, for example, provide a source of resistance genes that could be identified through proper profiling of accessions. Also some clones within "Apple" bananas are now known to be diploids while others are triploid (INIBAP, 2005). Morphologically, determining which ones are AAB and which ones are AB remains a question, and ploidy studies have been done to address this. Ploidy studies were also undertaken to identify the correct genomic composition of accessions.

Classification also helps standardize varieties and ensures that true to type varieties are sold in the market. Well classified varieties whose names are agreed

upon by everyone should enhance communication and the exchange of information among scientists and traders dealing with these bananas.

The following were the general objectives of my studies:

1. To use molecular and morphological tools to describe the variation that exist within the East African AAB "Sukari Ndizi" and AA "Muraru" dessert bananas and to permit the description of accessions found within the two groupings;
2. To classify AAB "Sukari Ndizi" and AA "Muraru" dessert bananas into distinguishable groups for ease of reference and communication purposes using various methods, and to assess the relative merits of these analyses;
3. To identify the key characteristics that describe the East African AAB "Sukari Ndizi" and AA "Muraru" dessert banana groups and to develop a provisional identification system for them. (This would facilitate the identification of accessions and possible synonyms in these groups.); and
4. To identify superior AAB "Apple" and AA "Muraru" cultivars for the farmers' use.

CHAPTER 2

LITERATURE REVIEW

2.1. General nomenclature systems for plants and cultivars

One of the major outcomes of a systematic study of nomenclature is the production of a predictive classification scheme that places related organisms together in order of their degree of relationship. For plants, this follows the International Code of Botanical Nomenclature (ICBN) that recognizes ranks, typically Kingdom, Phylum, Class, Order, Family, Genus, and Species. Over 40 sub-designations such as subfamily, superorder, or sub-species are also recognized. Cultivated plants are governed by the International Code of Nomenclature for Cultivated Plants (ICNCP) (Trehane *et al.* 1995) reflecting the need to recognize landraces, clonal variants, hybrids, and selected lines that are not recognized by the ICBN. Known hybrids are recognized in both codes by the convention of placing an X between the genus name and the specific epithet. The ICNCP also recognizes 'cultivar', 'group,' and 'graft chimera' (Brickell *et al.* 2004).

For cultivated bananas in the genus *Musa*, the focus is largely on the relationships of hybrid cultivars of *M. acuminata* and *M. balbisiana*. Variation is complex among the hybrid progeny making classification of cultivars difficult. Hetterscheid and Brandenburg (1995) felt that cultivated plants are not taxa in the same sense that the term is applied to wild organisms. They stated that classifying cultivated plants has different objectives from those of classifying groups of naturally occurring plants, and therefore need a 'special- purpose' classification. Classification

of the variation in crops is necessary for the same reasons that it is necessary for other organisms: to provide a useful framework for ordering the phenotypic diversity, while honoring the genetic relatedness of taxa, in this case, involving variants below the species level. Domesticated plants have challenged (Harlan 1992) conventional taxonomists because they cause huge contradictions when evaluated from a phenetic versus a phylogenetic viewpoint – the two facets of modern systematics. In crops, human selection has resulted in phenotypic differences that classical taxonomists would attribute to different species (or even different genera), yet from the biological standpoint, they are all one species, as they can freely inter-breed and produce fertile offspring. So infra-specific variation in crops is treated differently because, unlike the wild plants, the phenotypic diversity in cultivars is no longer closely linked to natural selection and speciation. Rather, variation is maintained (and amplified) by special inputs from agriculturists, and the number of genes involved in most phenotypic changes is small, so there is no loss of inter-fertility.

Many cultivars known today are also of complex hybrid origin. Through the use of new techniques in breeding from molecular biology, such as somatic hybridization (Feldman, 1983), some plant groups or cultivar classification cannot be linked with botanical classification at any *a priori* determined level. Also some of these novel hybrids may remain unidentified due to a lack of full disclosure of their origin. In these cases only detailed genetic investigations may provide reliable information on the origin and taxonomic status of these cultivars.

There exist many different systems of classification (Stace 1980); some differ only in detail but others in their broadest outline. It has not been found possible to incorporate all the good points of the various schemes into a single system of classification which also eliminates all the bad points (Stace 1980). Overall it is clear that the term 'taxonomic relationship,' often used very loosely, has a precise meaning only in so far as it is applicable to a particular system of classification. Two taxa may be closely related according to one system, but distantly so in another. Cultivated groups may be based on one or more user criteria, and an individual cultivar may belong to more than one group. These issues are especially relevant for crop plants, including bananas, where a number of informal categories have been introduced by various authors to cover the variation between cultivar groups and cultivars.

2.2. Historical approaches to taxonomy

Taxonomy is a discipline that deals with description, identification, nomenclature, and hierarchical classifications of organisms that follows a strict set of principles, procedures and rules (Sneath and Sokal 1973, Stace 1980, Simpson 2006). One purpose of classification is to provide a system for cataloguing and expressing the relationships of individual units which are referred to as taxa. Originally taxonomy only referred to this science of classifying living organisms (this is now known as alpha taxonomy). However, the term is now often applied in a wider, more general sense to a scheme based both on the classification of things and the *principles* underlying such a classification.

2.2.1. Linnean system

Within taxonomy, the names of organisms should be based on a universal and unambiguous system for ease of communication (see below for plant nomenclature systems). The formalization of a hierarchical system of nomenclature by Linneaus (1753) established a universal framework for describing and categorizing biological diversity. Within this context taxonomists often devoted years of study to particular groups of organisms. The Linnean system has also been called a "Sexual system," as it was originally based largely on characteristics such as the number of stamens on each flower, or other simple features of the stamens, such as their degree of cohesion or relative lengths (Stace 1980).

The simplicity of the Linnean system depended primarily on the use of single character. This would ensure that virtually anyone with minimum training could correctly identify a plant at least to its class and order. The Linnean system is also based on other morphological traits, e.g., cells tissues, or organs. This use of morphology relies on the selection of certain character states, the presence or absence of which group species together. It is also apparent, however, that there are some weaknesses this hierarchical system. With plants, for example, Linnaeus often used shared characters that arose independently by convergence or parallel evolution (Graur and Li 2000) rather than being inherited from a common ancestor (a phenomenon known as homoplasy. This system also often does not provide enough consistent rules to determine when a new genus (or family, order, etc.) is warranted. Finally, while the Linnaean system is hierarchical, it does not describe the

evolutionary or genetic relationships within a group, for example, all families within an order (Hillis and Moritz 1996).

2.2.2. Numerical taxonomy

To address these issues, some taxonomists since Linneaus' time came to believe that natural classification should be based on many characters. This led to an increase in the amount and breadth of data on which their classifications were based. One such approach *numerical taxonomy* was developed in the 1960s, based on *phenetics* or measurement of phenotype. In this approach, organisms were grouped and classified in terms of overall similarity as measured by defined rules using as many traits of the organism as possible. Their guiding principle was that the best classification would result from analyses based on many features or characters, with every character being afforded equal weight. Philosophically, numerical taxonomists aimed to develop methods that were objective, explicit, and repeatable, both in the evaluation of taxonomic relationships and in the erection of taxa. They used these to make quantitative estimates of phenetic similarities for correlating and grouping of taxa.

Numerical taxonomy which groups taxonomic units based on their character states (Sneath and Sokal 1973), embraces a number of fundamental assumptions and philosophical attitudes toward taxonomic work. The fundamental position of numerical taxonomy may be summarized as follows based on Sneath and Sokal (1973):

1. The greater the content of information in the taxa of a classification and the more characters on which it is based, the better a given classification will be.
2. *A priori*, every character is of equal weight in creating natural taxa.
3. Overall similarity between any two entities is a function of their individual similarities in each of the many characters in which they are being compared.
4. Distinct taxa can be recognized because correlations of characters differ in the groups of organisms under study.
5. Phylogenetic inferences can be made from the taxonomic structures of a group and from character correlations, given certain assumptions about evolutionary pathways and mechanisms.
6. Taxonomy is viewed and practiced as an empirical science.
7. Classifications are based on phenetic similarity.

2.3. Systematics

Systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them (Sneath and Sokal 1973). It includes traditional taxonomy, the science of naming and classifying organisms, and phylogeny, the evolutionary relationships among organisms (Simpson 2006). Systematics uses data from many sources, i.e., morphology, anatomy, embryology/development, ultrastructure, paleontology, ecology, geography, chemistry, physiology, genetics, and cell and molecular biology to obtain as accurate an assessment of relationships as possible.

The methodologies employed in systematic studies have varied with the types of information available and the development of theoretical bases for their application. Initially, methods were highly individual, developed by taxonomists in a particular group, and were largely subjective. As discussed earlier, this changed in the 1960s with the introduction of phenetic and numerical taxonomic methods. Phenetic methods describe an organization from objective measures of dissimilarities between the entities and refer the introduction of a genetic assumption to the interpretation (Perrier *et al.* 2003). The dissimilarities are estimated overall for all the characters observed. Today, relationships are established on cladistic methodology based on shared derived characters each representing a unique evolutionary event (Simpson 2006) shared by directly related taxa (monophyletic group). One goal of systematics is to understand the evolutionary history and relationships of a group of organisms. For this purpose systematics often uses phylogenetic reconstruction tool for addressing many biological questions on relationships of organisms. Phylogeny can also provide a scientific basis for defining or delimiting species and for establishing that one taxonomic unit is distinct from other closely related taxa. This is difficult to apply to cultivars where hybrids are the norm as there is no clear monophyletic grouping. A strict monophyletic grouping includes an ancestor and all its descendants, impossibility for hybrids created from two different monophyletic parental lineages. As a result phenetic methods are more suitable to cultivated plants such as bananas.

2.4. Character sets used in taxonomic and systematic analyses of bananas

2.4.1. Morphological characters

Traditionally, characterization and classification of bananas has been accomplished by the use of morphological descriptors (Simmonds and Shepherd 1955, Stover and Simmonds 1962). This method involves evaluation of genetic material based on observable phenotypes, such as fruit color, or on quantitative traits, such as bunch weight useful in describing cultivars. De Langhe *et al.* (2005) stated that Numerical Morpho-Taxonomy (NMT) was first used successfully in banana classification by Simmonds and Weatherup (1990). Karamura (1998) used NMT with success in the classification of the AAA East African highland bananas.

2.4.1.1. Choice of characters to use

It has been claimed that it is not possible to have absolute measures of resemblance because such measures would involve a random selection among the never-ending array of attributes (characters) of the organism. However, significant estimates of resemblance can be made once there is agreement on what characters are to be stated as relevant in the taxonomy (Sneath and Sokal 1973). Choice of characters forms a most important decision of any study concerned with identification and classification of organisms (Karamura 1998). How a classification of objects turns out will basically depend on the characters selected, how varied they are, whether they show discontinuities, and also the way they are treated (Pankhurst 1991). All kinds of characters from all parts of the body and all the stages of life cycles should be used (Sneath and Sokal 1973). And it is important to choose

polymorphic characters that vary in the study group (Sneath and Sokal 1973). Other criteria for choosing characters are ease of observation, availability (Karamura 1998), and the ability to easily differentiate the characters' states. A systematic survey of all known characters, or the inclusion of all characters the investigator has been able to observe, should minimize bias in classification that could result from using only a small number of subjectively chosen characters. Sneath and Sokal (1973) developed the *nexus hypothesis* which assumes that every phenetic character is likely to be affected by more than one genetic factor and that; conversely, most genes affect more than one character. The result is a complicated *nexus* of cause and effect. Any character should give information about several genes and it should be possible, in general to pick up the effect of a given gene through any one of several characters. There would be no *a priori* grounds for favoring one character over another; however, identical classifications are not produced from different sets of characters for the same OTUs (Sneath and Sokal 1973). It is argued that highly heritable characters also known as 'constant characters,' should be selected for classification because they remain relatively stable over a wide range of environmental conditions. Selection of characters to use is a matter of debate, but in morphological classification, it has been argued that as many characters as possible ought to be used (Sneath and Sokal 1973, Pankhurst 1991).

Morphological classification of bananas has made use of both qualitative and quantitative traits (Ortiz 1997, Karamura 1998, Nsabimana 2005). Ortiz (1997) found high heritability and little environmental effect in fruit size in bananas, such a descriptor for example, is useful in the characterization of banana accessions. It is

also important to understand the target group of bananas that one is dealing with to ensure that important traits bring out the differences within or between the study samples. Some of the morphological traits, such as those used by Nsabimana (2005) to characterize the Rwandan germplasm, are horticultural in nature and are also of interest to growers.

2.4.2. Ploidy level classification

The study of cytogenetics can and has been used for the purpose of classification of plants. The total number of chromosome per cell is used to determine the ploidy level of an organism. The application of cytogenetics to *Musa* has greatly improved the understanding of the chromosome structure and karyotype variation within the genus. The genome size should be constant within a population of interbreeding individuals (Lysak *et al.* 1999). However, the speciation of *Musa* has been accompanied both by geographical isolation and the frequent occurrence of asexual propagation, which could favor the diversification of genome size.

In plants, ploidy estimation is usually carried out by counting chromosomes on microscope slides prepared from actively growing root tips. The main limitations to the use of conventional chromosome counting include:

1. A very low frequency of dividing cells (the mitotic index in root tips may be 1% or less).
2. A rare occurrence of well spread metaphase plates suitable for chromosome counting.
3. Time needed to screen a sufficient number of samples (Novak 1992).

This method can also be used in *Musa*; however, it requires trained personnel and is generally complicated by the presence of very small chromosomes (Osuji *et al.* 1996) that are difficult to identify, making this method arduous and subject to error. The various phenotypic traits have been used as an alternative means for the estimation of ploidy level, including stomata size, stomata density, and pollen size. Unfortunately, due to the strong influence of genotype, these methods were not found reproducible and cannot be recommended for reliable ploidy estimation in *Musa* (Vandenhout *et al.* 1995, Van Duren *et al.* 1996).

2.4.2.1. Flow cytometry

Another indirect way of ploidy estimation commonly used is the flow cytometry method. Flow cytometry is a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid (Shapiro 2003). Modern flow cytometers can analyze several thousand particles every second in "real time" and can actively separate out and isolate particles having specified properties. This methodology is based on the use of DNA specific fluorochromes and on the analysis of the relative fluorescence intensity of stained nuclei. Because the DNA content of nuclei is related to the ploidy level, flow cytometric determination of DNA content may be used as an alternative to chromosome counting and other conventional methods for ploidy determination (Dolezel 1991, 1997).

2.4.3. Molecular classification

Due to the subjective nature of the classifications based on morphological character states, many of which are continuously distributed rather than discrete, scientists are increasingly embracing the use of molecular techniques in the classification and characterization of *Musa* groups. In the past few decades, the evaluation of protein variants, DNA markers, DNA sequences, and the order of genes along chromosomes, represents a transition from simple awareness of a relationship between phenotype and genotype, to plain study of genetic events responsible for taxonomic divergence (Peterson, 1997). The following molecular markers have been used in characterization studies of bananas.

2.4.3.1. Protein based methods

Isozyme or allozyme analysis was the first type of molecular analysis widely employed (Peterson 1997). Isozymes are proteins that have different molecular forms but catalyze identical reactions. Isozymes have been used to a great extent as molecular identification tags. They are generally expressed in a co-dominant fashion and rarely exhibit epistatic interaction (Tanksley and Rick 1980). The technique extracts water-soluble enzymes from plant tissue(s) which are then separated by size, charge, and structure by means of starch or acrylamide gel electrophoresis. Differences in charge, which affect the electrophoretic mobility of individual polypeptides, are the result of variations in the polypeptides amino acid sequence, which, in turn, reflect variations in the nucleotide sequences of the corresponding structural genes.

When the different molecular forms are separated on electrophoretic gels and the enzyme activity is visualized using appropriate straining techniques, a banding pattern is produced. This pattern is treated as a phenotype during data analysis. Genetic tests are frequently performed in order to determine which bands represent gene products transcribed for by allelic genes (allozymes). Isozymes have been used extensively as genetic markers for clonal identification (Gorman and Kiang 1977, Tanksley and Orton 1983, Weeden and Lamb 1985) in taxonomic and evolutionary studies (Gottlieb 1977) and to monitor the genetic stability of *in vitro* cultures (Brettell *et al.* 1986). This method does not sample genes that code for water insoluble or membrane-bound proteins, RNAs or genes with regulatory functions (Gottlieb 1971), which means that any variability in these genes is not captured. In addition, some isozymes are not developmentally stable and because of this, developmental effects must be identified and characterized prior to use.

Allozyme analysis has been used longer than other forms of molecular analysis; inclusion of previously identified markers allows comparison to this now classic work. The research of Rivera (1982) and Jarret (1986) demonstrated that enzyme polymorphism may be used to detect differences between *Musa acuminata* sub-species, genomic groups and between cultivars. However, Rueveni (1987) indicated that variation within the Cavendish sub-group could not be detected by enzyme polymorphisms. Lebot *et al.* (1993) used isozymes to determine the genetic relationships of a variety of banana accessions and was able to differentiate major groups and reveal patterns of association within groups. Allozyme analysis has the advantages of being relatively inexpensive and easy to perform, and requiring little

preliminary work but beginning in the 1980s, genetic marker analysis has shifted from the use of protein markers to DNA markers (Peterson 1997).

2.4.3.2. DNA based methods

2.4.3.2.1. Restriction Fragment Length Polymorphisms (RFLPs)

RFLP analyses provided the first successful application of DNA markers to bananas and plantain (Gawel and Jarret 1991, Jarret *et al.* 1992). In this procedure, genomic DNA is digested at DNA targets using restriction endonucleases that normally recognize six base-pair sequences. DNA, isolated from organelles such as chloroplasts and mitochondria or cell nuclei, digested with restriction endonucleases results in fragments of varying size nucleic acid that can be visualized using gel electrophoresis. Variation between individuals in the base-pair sequence of the DNA results in different cleavage sites and, therefore, different size fragments when each individual's DNA is digested. When these fragments are separated by size via agarose gel electrophoresis, they yield a distinct pattern that is representative of polymorphisms for the cleavage sites. RFLPs may be detected by Southern hybridization using various labeled DNA probes.

This technique has been the most widely used form of marker analysis (Peterson 1997) and it offers several advantages over the use of isozymes. Although laboratory techniques for RFLP detection are more complex, they are also more standard and are more sensitive in detecting polymorphisms (Bernastsky and Tanksley 1986) since they monitor a significantly greater portion of the total genome (Beckmann and Soller 1983). In contrast to isozymes, RFLPs detect differences in

DNA and not differences in gene expression or products of water soluble enzymes. In addition, a virtually unlimited number of probes and a large number of restriction enzymes may be used in various combinations to detect polymorphisms (Jarret 1990). RFLP markers are co-dominant, and scoring is simple. RFLP technique requires some sequence information and the use of probes. The main factors limiting the application of the technique is cost, and the fact that relatively few laboratories have the expertise, facilities, and germplasm to conduct this type of research. RFLP technique is also very labor intensive, is non selective for highly polymorphic DNA, and requires large initial DNA sample (Kaemmer *et al.* 1997).

2.4.3.2.2. Polymerase chain reaction (PCR)

The PCR is a theoretically simple technique that results in the exponential amplification of almost any region of a selected DNA molecule. It allows detection of specific DNA sequence against the background of a complex genome (Bloom *et al.* 1996). It works in a way that is similar to DNA replication in nature but employs selective amplification of a target region. This provides access to hundreds of thousands of copies of a particular gene or DNA region. PCR is a method where a specific sequence within a double-stranded DNA is amplified using primers flanking the region (Bloom *et al.* 1996). The PCR amplification has become an indispensable tool in a great variety of applications.

2.4.3.2.3. Random Amplified Polymorphism DNA (RAPD) markers

The key to RAPD analysis is low-stringency annealing of short (10 base pair) PCR primers of arbitrary sequence are utilized to direct amplification of DNA products of unknown sequence. RAPD markers were designed to solve the problem of a lack of pre-existing DNA sequence information. Unlike other PCR-based systems, in RAPD only one primer, a single, arbitrarily chosen short oligonucleotide is typically used. The short sequence of the primers takes advantage of a multitude of potential primer binding sites throughout the genome, but efficient amplification of DNA fragments may occur when two primer binding sites occur only in close proximity on opposite strands. DNA markers generated by RAPD have been widely used for genetic mapping, molecular taxonomy, and molecular diagnostics (Williams *et al.* 1990, Powell *et al.* 1995, Ren *et al.* 1995). This technique has been used to classify banana accessions (Onguso *et al.* 2004, Kahangi *et al.* 2002, and Uma *et al.* 2004).

The advantages of this approach include the limited investment of time and training required to get the technique running. Sets of several hundred primers are available commercially, and most informative markers can be selected. No genomic libraries or sequence information from the target species is required, and only small amounts of DNA are required. However, RAPDs are generally dominant in nature, and no more than approximately 5% are estimated to be co-dominant (Peterson 1997). Dominance is a major limitation to this method as polymorphisms are generally detected as the presence or absence of a particular band. Dominant markers are less informative than co-dominant ones. RAPDs also have inherently low reliability due

to low-stringency primer annealing, and the inability to discern differences in sequence homology among similarly sized fragments. RAPD analysis, although initially promising, has declined in usage as a result of these drawbacks and a lack of reproducibility in marker patterns.

2.4.3.2.4. Amplified Fragment Length Polymorphism (AFLP)

DNA analysis without prior sequence information can be potentially extremely useful. Vos *et al.* (1995) described the amplified fragment length polymorphism (AFLP) technique as a novel and very powerful DNA fingerprinting technique for analyzing DNA of any origin or complexity. The DNA finger-printing technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA and involves three steps: (i) restriction digestion of DNA and ligation of oligonucleotide adapters (ii) selective amplification of sub-sets of restriction fragments, and (iii) electrophoretic separation of the amplified fragments.

AFLP has many advantages. It provides a flexible method to survey a large number of restriction sites for polymorphisms without requiring cloned probes or sequence information. The use of longer primers promotes a more reliable amplification than RAPD analysis, selective nucleotides allow for the examination of a subset of potential markers, and 256 possible combinations of selective nucleotides allows examination of large numbers of potential markers. Disadvantages include a higher error rate, than RFLP and microsatellite analysis. In addition, the method is more complex than some other types of marker analysis requiring double digest, ligation, and two amplifications. Incomplete digests may also be a cause of false

markers. AFLP markers are also dominant, and have been used in bananas classification (Tugume *et al.* 200, Wong *et al.* 2002).

2.4.3.2.5. Microsatellite markers

2.4.3.2.5.1. Nuclei microsatellite markers

DNA microsatellites, known as Simple Sequence Repeats (SSR), are polymorphic elements that consist of a succession of small repeated motifs, usually less than four nucleotides long (Wang *et al.* 1994). These short tandem repeat elements are widely distributed throughout the nuclear genomes of eukaryotes (Tautz 1989, Ellegren 2000; 2004). Fragments containing microsatellites can be amplified by the polymerase chain reaction (PCR) by using a pair of primers flanking the repeat sequence. The polymorphism between genotypes is primarily due to the variation in the number of repeat units. In *Musa*, Crouch *et al.* (1998) have developed specific primers for 24 microsatellite loci, while Lagoda *et al.* (1998) also proposed primers for a further 47 loci. Studies by Kaemmer *et al.* (1997) demonstrated that SSRs are readily applicable to the study of *Musa* genetics. In contrast to an RFLP probe, which must be maintained in a vector, purified and labeled for genotyping, only the sequence information for a pair of SSR primers, which can be stored in a computer data base, is required. Also, SSR loci are uniformly distributed in the genome and their allele numbers tend to be very high, ensuring a high level of heterozygosity. Allelic differences are codominant, and consequently genotypes can clearly and easily be identified after PCR amplification and gel electrophoresis.

Microsatellite analysis have been demonstrated to be an excellent marker for banana fingerprinting (Grapin *et al.* 1998) as they are highly polymorphic, show a co-dominant mode of inheritance, are reproducible, and are easy to interpret. Microsatellites tend to be remarkably informative, apparently due to the ability of tandem repeat sequences to expand or contract during DNA replication (Peterson, 1997). Evidence in rice (McCouch *et al.* 1997) indicates that microsatellites are a good tool for discriminating between closely related accessions, and provide a valuable source of genetic markers to complement the use of RFLPs in genetic analysis and marker - assisted breeding. In support of long-term germplasm conservation, microsatellite markers have been usefully employed to demonstrate that accessions or cultivars are true to type, to help ensure proper maintenance (i.e. detect duplications, seed mixtures, inadvertent outcrossing, and genetic drift to determine the degree of relatedness among individuals or groups of accessions (Yang 1994, Olufowote 1997), to clarify the genetic structure or partitioning of variation among individuals, accessions, populations, and species (Wu and Tanksley 1993, Xiao 1996), and to help determine the presence of a specific gene or gene complex in a particular accession (Ayres *et al.* 1997, Blair and McCouch 1997, Koh *et al.* 1996, Xiao 1996a, Xiao 1996b). In Brazil, microsatellites were used (Creste *et al.* 2004) to investigate the genetic variability and relationships between 58 *Musa* genotypes, including 49 diploids and nine triploid cultivars. The use of microsatellite-derived markers in molecular breeding programs is facilitated by the relatively simple laboratory techniques involved (PCR, non-radioactive detection, potential for automated analysis) as compared to the analysis of RFLP markers. In *Musa*, the

flanking primer sequences have been developed (Kaemmer *et al.* 1997, Crouch *et al.* 1998, Lagoda *et al.* 1998), which is the most expensive part of the use of microsatellites. Microsatellites have not been previously applied to banana clones in East Africa and were used in this study because of the above advantages.

2.4.3.2.5.2. Chloroplast microsatellite markers

Microsatellite studies have also been done using chloroplast DNA. The presence of chloroplast DNA was established in 1963 (Masahiro 2003). The non-recombinant, uniparentally inherited nature of organelle genomes (Faure *et al.* 1994; Provan *et al.* 2001) makes them useful tools for evolutionary studies. The chloroplast genome is highly conserved (Palmer 1986), as it is slower evolving than nuclear DNA. The discovery of polymorphic mononucleotide repeats in the chloroplast genome (Provan *et al.* 2001) and the development of universal primers (Cheng *et al* 2006) have allowed analysis of population variation within species. The universal primers are highly conserved yet span a region that includes enough variation to be phylogenetically useful at the species level and below. Chloroplast microsatellites (cpSSRs) are similar to nuclear microsatellites but the repeat is usually only 1 base pair (bp), i.e. (T)n .

Most chloroplast microsatellites loci are of short length and have a limited number of alleles. The data generally consist of the alleles themselves. The use of capillary gel electrophoresis or genotyping is very useful in determining allelic differences in chloroplast SSRs, as it is able to detect very small size differences that cannot be clearly resolved in gel electrophoresis.

Bananas are unusual in that their chloroplast DNA is maternally inherited while their mitochondrial DNA is paternally inherited (Faure *et al.* 1994, Carreel *et al.* 2002). Vegetative propagation of bananas allows the preservation of a genotype arising from a cross that occurred hundreds of years ago, so chloroplast SSRs can reveal maternal parentage in banana clones involving interspecific hybridization between *Musa* species.

2.5. Methods for systematic analysis

2.5.1. Multivariate analyses

In most experimental studies, numerous different assessments and measurements may be made on each sample studied. These measurements may be of independent characteristics of the samples, but more likely they are different ways of measuring underlying characteristics of the material under investigation and, as such, are likely to be correlated. For example, the height and girth of banana are both measures of size and are likely to be positively correlated. Multivariate analysis is a term used to cover a range of statistical techniques for describing, simplifying, and analyzing data sets where many different variables are measured on a set of samples or objects (Roger *et al.* 2003), but usually with no prior differentiation of variables into causes and effects. We can look at any one variable in isolation but, to get the whole picture, the variables must be considered jointly. Most multivariate techniques are both descriptive and graphical.

2.5.2. Cluster analysis (CA)

Cluster analysis (CA) is a generic name for a group of techniques that place objects or units in groups or clusters in an "objective" manner, based only on similarities in the data rather than any a *priori* groupings (Roger *et al.* 2003). The aim is to produce inclusive groupings within which populations or individuals are relatively more similar to one another, based on similarity or dissimilarity measurements calculated from the data.

2.5.3. Association coefficients (Similarity measures)

Association coefficients are pair functions that measure the agreement between pairs of operational taxonomic units (OTUs) over an array of two-state or multi-state characters (Sneath and Sokal 1973). There are many association coefficients for both biological and non biological analyses (Sneath and Sokal 1973). Discrete characters coded in two, or in a few states, are particularly appropriate for the computation of association coefficients, although continuous characters can also be coded (albeit usually with a loss of information) to yield an association coefficient.

In any cluster analysis, deciding on how to measure similarity or closeness between the units for which groupings are required (Roger *et al.* 2003) is a significant task. Correlation coefficients are association coefficients used to measure similarity, and there are several available to choose from including "Simple Matching" and "Jaccard's" similarity coefficients.

The descriptors of genetic diversity are often binary variables that are represented as absence (0) or presence (1) of a character. For two individuals, *i* and *j*,

a is the number of markers that are simultaneously present in *i* and *j*. Similarly, *d* is the number of absences in common, *b* is the number of presence in *i* and absences in *j*, and *c* is the number of absence in *i* and presence in j. The general principle of construction of all the indexes is the same. However, the value does not have absolute meaning and must be reported on the basis of comparison (Perrier *et al.* 2003). The indexes differ in their mode of estimating the number of agreements and in the choice of the basis of comparison. The estimation agreement depends on the meaning assigned to the absence modality. If only modality 1 is considered informative, modality 0 expresses mainly an absence of information. If 0 and 1 are both considered informative and can be considered as two modalities of a qualitative variable, then the number of agreement is $a + d$, the number of presences and absences in common. The choice between these two attitudes depends entirely on the nature of the characters analyzed. Regarding genetic markers of diversity, it is clear that biological knowledge of the markers being considered is needed to enable us to choose the most appropriate model. One approach, the Jaccard index, uses the basis of comparison as the number of presences found in *i* or in *j*, $a / a + b + c$. With the Jaccard index, *a* is compared to the number of variables, but the double absences are treated as missing data. Another approach, the simple matching index, considers both the common presences and common absences of markers in *i* or in *j*, thus $a + d / a + b + c + d$. In this study both the Jaccard's and Simple Matching indexes were used; these may sometimes give different cluster solutions.

When character states are compared over pairs of columns in a conventional data matrix, the outcome can be summarized in a conventional 2X2 frequency table e.g.:

		OTU_i	
		1	0
OTU_k	1	**a**	**b**
	0	**c**	**d**

2.5.3.1. The coefficient of Jaccard (Sneath)

The coefficient of Jaccard omits consideration of absences.

$S_j = a/a+u = a/a+b+c$

2.5.3.2. The Simple Matching Coefficient (Sokal and Michener 1958)

Simple matching coefficient considers both shared presences and absences.

$S_{sm} = a+d/ a+b+c+d$

For molecular markers, the genotype resemblance between two individuals can be measured by a genetic similarity defined from allele frequencies. Microsatellite markers generally give direct genetic information and allow coding in alleles by identification of the allele composition of each locus (Perrier *et al.* 2003). The genetic similarity, as defined here can thus be directly estimated; the result is less loss of information.

2.5.4 Phylogenetic trees

In phylogenetic studies, the evolutionary relationships among a group of organisms can be shown by means of a phylogenetic tree or dendrogram (Graur and Li 2000). A phylogenetic tree is a graph composed of nodes and branches, and in which only one branch connects any two adjacent nodes. The nodes represent taxonomic units. The taxonomic units represented by the nodes can be species (or higher taxa), populations, individuals, or genes. The branches define the relationships among the taxonomic units in terms of descent and ancestry.

Both molecular and morphological data can be used in phylogenetic tree construction. However, only one of all the possible trees that can be built represents the "true" phylogenetic tree. A tree achieved by using a set of data and a method of tree reconstruction is called an inferred tree, and this may or may not be identical to the "true" tree. Inferring a phylogeny is an estimation procedure, based on incomplete information (Graur and Li 2000).

2.5.4.1 Constructing phylogenetic trees

Because many different phylogenetic trees can be produced from any set of OTUs, we must specify criteria for selecting one or a few trees as representing our best estimate of the true evolutionary history. Several methods of tree reconstruction employ a specific sequence of steps (i.e., an algorithm) for constructing a tree. This class of methods combines tree inference and the definition of the optimality criterion for selecting the preferred tree into a single statement. Numerous methods have been proposed in the literature (Sneath and Sokal 1973, Felsenstein 1982). Criteria for

choosing a method to use include its power to identify the correct tree, consistency and robustness in identifying the correct tree even when some of the assumptions of the method are in error (Daniel 2000). Many authors choose to analyze their data using multiple methods in hope that the resulting trees will differ (at most) in non-essential details (Daniel 2000).

One of the most commonly used methods are the "Distance methods", based on the pairwise differences between the character states. The two most commonly used are unweighted pair group method using arithmetic mean (UPGMA) and neighbor joining. The UPGMA is favored because it assumes a constant rate of evolution in each branch, however, if this is violated, the approach performs poorly. Neighbor joining (Saitou and Nei 1987) is the second distance method. It sequentially groups the most closely related pairs of character states. This method is recommended for being extremely efficient method of reconstructing phylogenetic trees.

Distance methods are typically phenetic approach (Graur and Li 2000). In this study the distance method UPGMA was used. The UPGMA method was originally developed for constructing taxonomic phenograms, i.e. trees that reflect the phenotypic similarities among OTUs (Sokal and Michener 1958), but it can also be used to construct phylogenetic trees if the rates of evolution is assumed to be approximately constant among the different lineages so that an approximate linear relation exists between evolutionary distance and divergence time (Nei 1975). The details of algorithm calculations can be found in Graur and Li (2000). UPGMA employs a sequential clustering algorithm, in which local topological relationships are

identified in order of decreasing similarity, and the phylogenetic tree is built in a stepwise manner. In this method similarity (dissimilarity) between an OTU and an established cluster is the average similarity (dissimilarity) of that OTU with all OTUs in the cluster. The unweighted method gives equal weight to each OTU within a cluster (Sneath and Sokal 1973, Panchen 1992).

2.5.4.2 Statistical significance of similarity coefficients

After inferring a phylogenetic tree, two questions may be asked: (1) How reliable is the tree or, are parts of the tree reliable? And (2) Is one tree significantly better than another tree? There is no direct way to test the accuracy of a phylogenetic tree, but, by using various techniques, it is possible to hypothesize the reliability of specific branching positions and sequences within a tree. The question "Is the difference in the resemblance of 2 OTUs significant when compared on separate set of characters?" is dealt with in numerical phenetics by two general types of optimality criteria: (1) Classification is optimal if it represents as closely as possible the original similarity matrix among the OTUs. (2) Certain arrangements of OTUs are good because specified measurable properties of their similarity coefficients are optimized (Sneath and Sokal 1973).

The most commonly applied best-fit method is the cophenetic correlations developed by Sokal and Rohlf (1962). This method assigns a Cophenetic Value to the similarity between pairs of OTUs implied by a given dendrogram and generates a matrix of such values for any set of OTUs. Cophenetic Value between any 2 OTUs is the maximum similarity implied by the dendrogram. A product moment correlation

coefficient is then compared between the elements Sjk of the original similarity matrix S and cophenetic values Cjk of the matrix C.

This cophenetic correlation coefficient (rcs) between the similarity values implied by the phenogram and those of the original matrix is obtained. Farris (1969) found that the UPGMA will always give the maximum cophenetic correlation coefficient. Cophenetic value is considered to be a satisfactory measure of the agreement of a phenogram with a similarity matrix (Sneath and Sokal 1973) and was used in this study.

Bootstrapping is another statistical method for placing confidence limits on a set of observations. Since its introduction into phylogenetic studies by Felsenstein (1985), it has frequently been used to estimate the confidence level of phylogenetic hypotheses. Bootstrapping belongs to a class of methods called resampling techniques (Graur and Li 2000), because it relies on repeated resampling of the original sample data set. The bootstrap values are expressed as percentages are indicated on the internal branches defining the clades, and are usually interpreted as confidence levels for the clades.

2.5.5. Principal component analysis (PCA)

Principal component analysis (PCA) is a multivariate technique used to find associations among variables and, in taxonomy, among specimens without an *a priori* subdivision of samples into discrete groups. It is a method of data reduction designed to clarify the relationships between two or more characters and to divide the total variance of all characters into a limited number of uncorrelated new variables (Iezzoni

and Pritts 1991). PCA seek out axes of maximum variation, referred to as "principal components." The first principal component (PC) usually summarizes more of the variability than any other variable. The second summarizes additional variability not summarized by the first and is uncorrelated with the first, and so on. Thus the total variation of a population may be broken down into components, each of which may say something about the size, shape, or some other quantitative aspect of the members of the population. The number of components depends on the number of original variables. Principal component analysis may also reveal the major underlying sources of variation and allow the data to be plotted in a succinct way, as well as to interpret or explain the multivariate system meaningfully. Results of principal component analysis are presented as scatter diagrams (two dimensional scatter plots). The observer assesses whether points fall into distinct constellation (Sneath and Sokal, 1973).

While the purpose of cluster analysis is to construct groups of individuals based on their overall similarity, PCA provides a representation of the same data but with the number of dimensions reduced. One may then be able to distinguish groups that were not obvious in the cluster analysis. The PCA is sometimes preferred, because it sometimes gives some indication as to why some objects cluster together. However, the principal-component scatter plot is only appropriate when a large proportion of the total variability in the data matrix can be explained by two or three principle components. In contrast, the CA approach will attempt to provide a solution regardless of the underlying dimensionality of the data (Roger *et al.* 2003).

2.6. Natural history and general descriptions of bananas

Bananas are best suited to warm, tropical climates. They are not tolerant to frost, but in appropriate climates can be grown from sea level to 2000m in elevation. Almost all banana cultivations fall within 30^0 latitude N and S of the equator (Simmonds 1966). Where water is not limiting, the rate of growth and time for fruit maturity is determined primarily by temperature. The optimum temperature for foliar growth is between 26^0 C and 28^0 C, and 29-30^0 C for fruit growth (Stover 1987). Bunch size has been reported to be optimal with a pseudostem temperature near the growing point of 21-24^0 (Stover 1987). Because of this dependence on temperature, the growth of different parts of the plant may vary even within the same clone in the same production unit.

Next to temperature, rainfall determines where most bananas can be produced. The banana plant is very sensitive to water deficiency (Stover 1987). When the deficiency becomes severe, older leaves fall prematurely. As long as moisture is adequate bananas grow well in full sun. In the tropics where bananas can be grown without irrigation, all months should have at least 100mm of rainfall (on average). In all other areas, irrigation is required if optimal production is to be achieved. Some species (notably *M. balbisiana*) can withstand weeks of dry weather in good condition, and the two sub-species of *M. acuminata* (*siamea* and *burmannica*) are also fairly drought tolerant when compared to others (e.g. *malaccensis* and *banksii*).

2.7. The origin of bananas in East Africa

Simmonds (1962) speculated that triploid bananas derived from *M. acuminata* came from Indonesia (Malaysia) to East Africa via Madagascar about 500AD, followed later by hybrid triploids between *M. acuminata* and *M. balbisiana*. From there they spread up the great lakes and to the west coast of Africa (Rowe and Rosales 1996). Champion (1970) suggests that most of the numerous cooking and beer cultivars in East Africa are descendants of one ancient introduction, and that their differences are due to successive mutations. East Africa (to the West and North of Lake Victoria) is regarded as a secondary centre of diversity for bananas (Stover and Simmonds 1962). Practically all the groups of the genus *Musa* are represented in Africa.

It is generally agreed that bananas of section *Musa* originated in south-East Asia, but Africa has contributed to *Musa* diversity by enriching it with two secondary lines of diversification namely, plantain and East African highland bananas. While the dates and routes of banana introductions from their native centre of diversity in South East Asia remains a subject of speculation (Karamura, 1998), the East Africa region hosts distinct types of bananas (INIBAP, 1998).

Baker and Simmonds (1951) noted that the great majority of the East African varieties are of *M. acuminata* origin and only a few were of hybrid (between *M. balbisiana* and *M. acuminata*) origin (e.g., the cosmopolitan silk fig of the West Indies which appeared in Zanzibar, Amani, and Moshi as Kipukusa or Kibungala; and the Mysore banana in Zanzibar as Kikonde). Baker and Simmonds (1951 and 1952) recorded the names of the various bananas in East African countries during a tour of

Kenya, Uganda, Tanganyika, and Zanzibar. Of interest to this study, the following names were documented during this expedition (Baker and Simmonds, 1952): Muraru Gatumia, Sukari (Nyanza and Uganda), and Guindi (Kiambu); Kipukusa cha Java at Amani and Kisukari at Moshi were thought to be similar to the Guindy of the Imperial College of Tropical Agriculture (ICTA). In addition the Kipukusa in Zanzibar was thought to be similar to the Silk fig (of the West Indies), and Safet Velchi of Bombay. The Kisubi was recorded in Buganda in Uganda as a recent introduction, although it was by then accepted and commonly grown.

2.8. Classification of the genus *Musa*

Based on the International Code of Botanical Nomenclature (ICBN), banana plants belong to the sub-kingdom *Tracheobionte* (vascular plants), the super division *Spermatophyte* (seed bearing plants), the division *Magnoliophyta* (flowering plants), the class *Liliopsida* (monocotyledons), the sub-class *Zingiberidae*, the order *Zingiberales*, the family *Musaceae*, and the genus *Musa*. Using morphological characters and chromosome numbers, Cheesman (1947) divided the genus *Musa* into four sections, namely, *Eumusa*, *Rhodochlamys*, *Callimusa*, and *Australimusa*; the use of this classification is still widespread. A new section, *Ingentimusa,* was subsequently created (Argent 1976) for species that did not conform to any of the existing four sections. Wong *et al.* (2002), using AFLP markers to characterize species in genus *Musa,* suggested reducing the five sections to three by combining sections *Rhodochlamys* and *Eumusa,* both having $x = 11$ chromosomes, into a new section *Musa* and absorbing *Australimusa* into *Callimusa,* both having $x = 10$

chromosomes. In Wong *et al.* (2002) proposed classification, *Ingentimusa* with x = 14 chromosomes is retained for *Musa ingens* as the third section. The authors obtained groupings that were well correlated with chromosome numbers and argued that there was no justification in their molecular analysis for the separation of *Musa* into five sections. Table 2.1 shows the old and new classifications of *Musa* sections.

Species in the section *Musa*, specifically those formerly placed in *Eumusa,* are of primary importance in agriculture for fruit, vegetable, and fiber production, among other uses (Table 2.1). The principal domesticated cultivars of banana (primarily used for fresh consumption) and plantain (starchy fruits used for cooking) are derived from one or both of two partially cross-compatible species, *Musa acuminata* and *Musa balbisiana* (Stover and Simmonds, 1987; Simmonds and Shepherd 1955). The joint distribution of *M. acuminata* and *M. balbisiana* by and large coincides with the overall distribution of the genus *Musa*; four outliers (*M. acuminata* in Pemba, Samoa, and Hawaii, and *M. balbisiana* in Hawaii) were treated as incidental to the main distributions (Simmonds 1962) and all four were described as not indigenous to the areas where they were found. The occurrence of hybrids between the two species may have resulted from the human introduction of edible *acuminata* into *balbisiana* populations (Simmonds 1962).

Table 2. 1: Old and new sections of important wild *Musa* species

Old section	New section	Species	Genome label	Distribution
Austalimusa[1] (Fibre, fruit*)	Callimusa[1]	*Lolodensis*	T	N New guinea, Halmahera,
		textilis		*Queensland to the Philppines
Callimusa (ornamental*)		*Maclayi*[2]		New guinea, New Ireland, Solomon
		peekelii[2]		*Indochina and Indonesia
		beccarii	T	
Eumusa (Fruit, fibre, vegetable*)	Musa	***acuminata***[2,3]	A	Sri Lanka, India, mainland and island SE Asia, SW Pacific, Australia[4], E India, Sikkim, S China, Philippines, E New guinea New Britain *South India to japan and Samoa
		balbisiana	B	
Rhodochlamys (ornamental*)		*ornata*		*India to Indonesia
		velutina		
		laterita		
		sanguinea		
Ingentimusa	Ingentimusa	ingens	-	-

Source: Argent 1976; Daniells *et al.* 2001; Jarret *et al.* 1992; Simmonds 1962; Wong *et al.* 2001
[1]Species affiliations of edible clones (Fe'I bananas) uncertain; multiple parentages likely.
[2]Multiple sub-species. [3]See Table 2.3, 2.4 Outliers (Polynesia and Pemba) are human introductions
(More important species in bold) *Simmonds 1966, Bartos *et a*l. 2005.

Cheesman (1948) stated that Colla was the first to recognize *M. acuminata* and *M. balbisiana* as wild species distinct from any other. Within *M. balbisiana*, genetic variability is small (Simmonds and Weatherup 1990, Uma *et al.* 2006), however, greater diversity has been described within *M. acuminata* which has diversified into a complex of several sub-species and many pathernocarpic landraces (Lysák *et al.* 1999, Ude *et al.* 2002). Although there is still no consensus among researchers on the number of sub-species within *M. acuminata*, at least 9 sub-species have been described (Table 2.2). The A genome is present in all cultivated bananas, and more

than one *M. acuminata* sub-species is involved in the origin of cultivated banana (Grapin *et al.* 1998, Ude *et al.* 2002). The studies of Ude *et al.* (2002) and Uma *et al.* (2006) showed that there is also much more genetic diversity within *M. balbisiana* than had previously been thought. In their study with RFLPs, Ude *et al.* (2002) found that eight accessions of *M. balbisiana* formed two sub-clusters, while Uma *et al.* (2004), using RAPDs, separated Indian *M. balbisiana* into four clusters and concluded that these were distinct sub-species.

Table 2. 2: The geographical distribution of wild *Musa acuminata* sub-species

Sub-species	**Distribution**
Banksii[1]	New Guinea, Manus, N Australia
burmannica	E India, Burma, Thailand, Sri Lanka
burmannicoides	E India, Burma, Thailand
errans[2]	Philippines
malaccensis	Malay Peninsula (lowlands), Thailand
microcarpa	Borneo
siamea	Thailand, Malay Peninsula, Vietnam
truncata	Malay Peninsula (highlands)
zebrina	Indonesia

Source: Argent 1976, Grapin *et al.* 1998, Lysák *et al.* 1999, Daniells et al. 2001, Valmayor 2001, Ude *et al.* 2002. [1]Raised to species rank by Argent (1976) and others. [2]Genetically distinct from M. *banksii* (Carreel et al. 2002), but morphologically similar; sometimes confused in the literature (Lebot 1999).

2.8.1 Genetic morphological characters used in bananas

Morphological characters have played a vital role in banana classification. A variety of methods that use morphological traits such as variations in leaf color, plant height and bunch shape have been used in banana studies. For characterization in banana, morphological characters were the first to be developed and optimized; 119 descriptors were defined to describe *Musa* germplasm in general (IPGRI 1999). Simmonds and Shepherd (1955) classified triploid cultivars into genetic groups based on a scoring method that indicates the relative contributions of the A and B genomes of the two wild species to the constitution of any given cultivar. By using the 15 characters (Table 2.3), each of which was to some degree diagnostic of differences between *M. acuminata* and *M. balbisiana*, they showed that the relative contributions made by the two wild species could be clearly discerned (Simmonds 1966). For each character in which the variety agreed with wild *M. acuminata*, the score of 1 was given; for each character in which the variety agreed with *M. balbisiana*, the score of 5 was given. Intermediate expressions of the character were assigned a score of 2, 3 or 4 according to its intensity. The scoring technique provides for a range of total scores from 15 (i.e., 15x1) for *M. acuminata* to 75 (i.e., 15x5) for *M. balbisiana*. The wild banana plants were shown to agree reasonably well with this expectation (Simmonds and Sherpherd 1962). A clone, it was argued, should then correspond with *M. acuminata* if it was derived solely from that species; it should have a proportionally larger score if *M. balbisiana* had made a contribution to its origin. In the case of polyploidy hybrids that evolved from the two natural *Musa* species, triploids, and a few tetraploids in various genome combinations, the picture is rather

complicated. The variation within the *M. acuminata* and *M. balbisiana* genomes (Grapin *et al.* 1998, Ude *et al.* 2002, Uma *et al.* 2006) also complicates the morphological interpretations of parental contribution.

Table 2. 3: Morphological difference between *Musa acumunata* and *Musa balbisiana*

Characters	***Musa acumunata***	***Musa balbisiana***
Pseudostem color	Brown or black blotched	green-yellow
Petiole canal margins	spreading	incurved
Peduncle	hairy	glabrous (smooth)
Inflorescence orientation	oblique to pendulous	sub horizontal to oblique
Pedicels	short	long
Fruits	dark green and non-waxy	pale green and waxy
Ovules	two rows of ovules in each loculus	four rows of ovules in each loculus
Male bud and bracts curling	slightly waxy, narrowly ovate, bracts roll back after opening* (convolute)	waxy, broadly ovate, bracts lift, but do not roll (imbricate)
Bract shoulder	Usually high (ratio<0.28)	Usually low (ratio>0.30)
Bract color	bright red to deep violet outside, light red to yellow inside	various shades of purple outside. scarlet inside
Bract scars	prominent	Scarcely prominent
Bract apex	Acute	Obtuse
Male flower color	cream, yellow, or orange with no pink flush	cream with a strong pink flush
Stigma color	Orange or rich yellow	Cream, pale yellow or pale pink
Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
Compound tepal	Same length as free tepal	twice as long as free tepal

Source: Simmonds, 1966.

2.8.2. Classification of cultivated bananas

As mentioned earlier, most cultivated bananas are natural hybrids of *M acuminata* and *M. balbisiana* that include various combinations of a broad spectrum of genomic groups ranging from diploids (AA and AB) to polyploids (AAA, AAB,

ABB, AABB, AAAB, and AAAA) (Table 2.4). Because several M. *acuminata* and *M. balbisiana* sub-species are involved in the hybridization of the cultivars, cultivated bananas are highly variable in genomic composition and ploidy level, and can be strikingly different morphologically. Cheesman (1948) recognized three groups of morphologically distinct cultivars from these hybrids. The first showed predominantly the botanical characters of *M. acuminata*; the second primarily exhibited the morphological features of *M. balbisiana*, and the third possessed a blend of morphological characters from these two wild species. Thus a number of cultivars were discovered that did not fit into the two Linnean species. Cheesman (1948 and 1949) attempted to apply Linnean nomenclature to the domesticated hybrid clones; the result was that he labeled the AAB group of plantains *Musa paradisiaca* and the AAB Silk dessert banana *Musa sapientum.*

The taxonomy of the banana hybrid cultivars was first studied by Simmonds and Shepherd (1955) using 15 morphological characters that differentiate *M. acuminata* and *M. balbisiana* (Table 2.3). The current classification of cultivated bananas is still based on the framework set by Simmonds and Shepherd (1955), as the cultivars cannot fit into the strictly hierarchical classification system 'the taxonomic hierarchy', since at the cultivar level, the variation is so complex and multidirectional and is no longer hierarchical (Heywood 1986). Polyploidy complicates the picture as it is necessary to know the ploidy of a clone before it can be satisfactorily classified (Simmonds 1966).

Table 2. 4: Conventional grouping of the cultivated Musa section

Section	***Musa***		
Species/hybrids	*M. acuminata*	*M. balbisiana*	*M. acuminata* x *M. balbisiana*
Wild type diploid	-	-	-
Diploid cultivars	AA	-	AB
Triploid cultivars	AAA	-	AAB, ABB
Tetraploid cultivars	AAAA	-	AABB, AAAB*, AABB*

Source Simmonds and Shepherd 1966 *Rowe and Rosale 1996 –artificial hybrids

The ICNCP provides for two categories of cultivated plants, the 'cultivar,' and the 'group' (Brickell *et al.* 2004) in Articles 18 to 20 and also deals with the formation of names of intergeneric graft-chimaeras at generic and cultivar levels in Article 21. The classification of cultivated bananas (Simmonds and Shepherd 1955) below the level of genus is based on genome groups. Many authors (Purseglove 1975, Stover and Simmonds 1987) provide for categories of group, subgroup, and clones. A group in the banana crop includes all clones at the same ploidy level that share the same genomic formula (e.g., AAA) but the ICNCP codes states that the cultivar group epithet must be a word or phrase, not a formula. What this means is that the cultivated banana classification breaks this rule in the ICNCP of the group epithet word. Simmond (1966) scoring method, rooted on 15 morphological characters of both *M. acuminata* and *M. balbisiana* cultivar analysis identified 7 groups: 2 diploids, 3 triploids and 2 tetraploids (Table 2.4). However, many differences exist even within these groups, partly due to the contributions of A and B

genomes from various wild sub species and partly due to mutation (Karamura 1998) that has contributed significantly to the variation of bananas (Simmonds 1966). As a result, different researchers (De Langhe and Valmayor 1980, Swennen and Vuylsteke 1987, Swennen 1988 and Lebot *et al.* 1994, Karamura 1998) have proposed various classifications below the genome group level that could be called sub-groups. A sub-group is an informal category used to differentiate clones within a genome group (e.g., AAA includes AAA dessert and AAA East African highland bananas).

Stover and Simmonds (1987), and Labot *et al.* (1994) came up with names for taxa within clones, morphotypes and cultivars and they agree that these probably arose by mutation. De Langhe (1961) hypothesized that "groups" of banana are derived from a single clonal source, but are greatly diversified by somatic mutation resulting in substantial variability. Simmonds (1966) noted that it is sometimes convenient to distinguish collectively a set of clones related to each other by a bud mutation from a single original clone, e.g., *Musa* (AAA group) Cavendish subgroup 'Robusta.' The classification of the *Musa* AAA group into three subgroups namely: Cavendish, Gros Michel, and Green Red was proposed by Cheesman (1933) based on morphological traits. The Cavendish clones are thought to have been derived from a single clone which diversified by somatic mutation (Daniells 1990). Stover and Simmonds (1987) grouped the Cavendish mutants into four: Dwarf Cavendish, Giant Cavendish, Grand Naine and Lacatan. The ranks of these are still unclear. Shepherd (1957) proposed a fourth subgroup in the *Musa* AAA group for the Lujugira-Mutika clones of the East African Highland region. Sebasigari (1987) differentiated the beer and the cooking clones amongst the Lujugira-Mutika subgroup but never gave them

named rankings. Karamura (1998) proposed a fourth category 'clone set' after sub-group and grouped the Lujugira-Mutika bananas into five 'clone sets'. The AAB group of bananas has been classified to sub-groups level as well (De Langhe and Valmayor 1980, Swennen and Vuylsteke 1987, Swennen 1988 and Lebot *et al.* 1994).

2.8.2.1. Classification of interspecific hybrid *Musa* AAB group

The interspecific triploid hybrid *Musa* AAB group was first identified by Cheesman (1948) as *Musa paradisiaca*, a plantain. A major concern about the original classification was the hybrid nature of this taxon. Nevertheless according to ICNCP rules, hybrids can be given scientific names; the epithet though must carry the prefix X to indicate the hybrid nature of the species. In the case of hybrid banana cultivars, *Musa* X *paradisiaca* Linn., the earliest published binomial should be used for all products of crosses between the two wild diploid species, their genome composition (i.e. AB, AAB, ABB) notwithstanding. Still, when Silk AAB was first encountered, it was named *Musa sapientum* as it was considered a separate species. The two AAB cultivars of *M.* X *paradisiaca* differ greatly in morphology and in how they are consumed. Plantain is cooked (boiled or roasted) and Silk is a dessert banana eaten raw. There are also the diploid AB types, like Safet Velchi and Kisubi that would both be grouped with the AAB bananas under *M.* X *paradisiaca* if the ICBN rules were to be followed. However, from what is a now known, plantains and silk bananas probably originated from different crosses of M. *acuminata* and *M. balbisiana* sub-species, making them of different genetic composition. The Nomenclature to group level only thus remains deficient.

The *Musa* AAB group has been classified into subgroups and clones (De Langhe and Valmayor 1980, Swennen and Vuylsteke 1987, Swennen 1988 and Labot *et al.* 1993 and 1994, Bakry *et al.* 2001). Examples of these subgroups are Plantain, Popoulu, Maia Maoli, Mysore, Silk, Pome and Pisang Raja. The plantains were further divided into French (with persistent male bud) and Horn (with no male bud) (Simmonds 1966) and the Horn subdivided further into three as the French horn, the False Horn and the Horn types (Tezenas du Montcel *et al.* 1983). Maia Maoli and Popoulu were divided into clones and morphotypes (Lebot *et al.* 1994). The available data do not make it clear what should be the placement of the AAB "Sukari Ndizi" dessert banana of East Africa. Only recently was it recognized that this subgroup is not a diploid AB, but a triploid AAB (INIBAP 2005).

2.8.2.1.1. The AAB "Apple" dessert banana sub-group

The small fruited AAB "Apple" dessert banana sub group is composed of diverse natural interspecific hybrids of *Musa acuminata* and *Musa balbisiana.* These are called Mysore, Silk, Prata, Pome, and Pisang Raja (Simmonds 1966, Stover 1987, Rowe and Rosale 1996, Bakry *et al.* 2001). These subgroups are probably hybrids of various combinations from different sub-species of the two *Musa* species. There are also tetraploid hybrids that have been developed, e.g., FHIA 1 from Prata ana (Rowe and Rosales 1996). The "Sukari Ndizi" of East Africa is within the AAB Apple subgroup.

2.8.2.2. Classification of intraspecific hybrid *Musa* AA

To my knowledge, the cultivated diploid AA bananas have not been fully classified. However, Daniells *et al.* (2001) have placed some of the cultivated AA bananas into 4 sub-groups, i.e., (1) Sucrier, e.g., Pisang Mas, (2) Pisang Lilin, e.g., Pisang Lilin, (3) Inamibal, e.g., Inamibal, and Pisang jari buaya, e.g., Niukin. Simmonds (1962) also recorded several distinct populations within *M. acuminata* namely macrocarpa, siamea, malaccensis, banksii, and burmannica, with outliers in Hawaii, Pemba, and Samoa. Simmonds (1966) stated that most edible diploids evolved entirely from the wild *M. acuminata* AA genome by becoming seedless. Additionally, several sub-species and many parthenocarpic landraces (Lysak *et al.* 1999, Ude *et al.* 2002) have been recognized within *M. acuminata*. The various origins of cultivated AA diploids, which are themselves basic to the evolution of the triploid cultivars, can only be speculative at the present time (Shepherd 1990). Efforts to classify *M. acuminata* into sub-species are difficult because of the occurrence of hybrids of *M. acuminata* (natural and artificial) and other sympatric sub-species. Since the AA genome is a variable species with several different sub-species (Simmonds and Weatherup 1990), the different edible diploids may have originated independently and more than once. Apart from being seedless, the edible diploids remain within the morphological range of their wild parents (Pickersgill 1986). It is possible that crosses between the different *M acuminata* sub-species produced some of the cultivated diploid AAs resulting in the variation of the diploid AA found in different geographical regions such as Papua New Guinea (PNG) and East Africa. Mutation may have added to the diversity seen in these regions. The

diploid bananas, wild and cultivated, are presently much less widespread than the cultivated triploids (Jenny *et al.* 2003). Jenny *et al.* (2003) noted that despite the large number of cultivated diploid clones known, no further subgroups have been established.

The current classifications of bananas are only as complete as the information available when they were constructed. All banana taxonomic groupings, however, accept Simmonds (1962) genome groups. Many accept the different groups and subgroups, but there is doubt about different taxa at the level of subgroup and below.

2.9. Horticultural evaluations

Horticultural traits are characteristics that include yield, storage quality, taste, color, shape, and size. Horticultural trait evaluation is important for identification of cultivars that are superior and that can be recommended to growers. These traits have also been used for characterization purposes of various crop species (Nsabimana 2005, Kim and Ko 1997) and native cultivars are sometimes even named according to the fruit shapes because these are important commercially. Horticultural traits can be changed by breeding to suit the desires of a target user group, or to enable a crop to withstand specific growing environmental conditions.

Development of agriculture depends on the development of crops that can be cultivated, harvested, and stored reliably. Productivity and quality of crop have been studied and improved since the start of organized agriculture many thousands of years ago. Plants like other living organisms, have genetic potential due to the genotypic makeup, and there is variation even between cultivars that is due to the genetic

composition. There is also variation between cultivars that is brought about by environment; some cultivars due to their genetic potential react differently to different environments. It is, therefore, important when doing evaluations to compare different cultivars of the same crop to evaluate them in a similar environment in order to separate out the variation due to environment from that due to genetic makeup. Horticultural studies that try to capture the genetic potential of cultivars include yield studies, efficient utilization of resources like water and fertilizer, resistance to pests and diseases, tolerance to environmental conditions such drought and temperature range, yield, and quality-related character studies, such as plant height, girth, fruit shape, size, color, weight, skin thickness, and shelf life. Selection is based on the utility of the crop to the grower, whether the crop is grown for commercial purposes or for domestic use and is performed on a wide range of traits at one time with some traits more heavily than others.

Variation between individuals is the raw material for plant improvement and if plants differ in phenotype, they are likely to have a different genetic makeup. One strategy used in crop improvement is the identification of a desirable variant in the population being evaluated. This variant may have horticultural characteristics that are of interest such as resistance to disease. The identification of polyploids that result in a higher yield because of their genetic makeup has also added to the selection of cultivars.

The largest percentage of cultivated banana is triploid. The most of the banana breeding work has been targeting the European and American markets, so most breeding and selection has been to meet the needs of the export market. Little effort

has been spent on bananas for local needs, however, some work has been done in Brazil to improve the dessert bananas AAB 'Prata' ('Pome') and 'Maca" AAB Silk and AA diploid (Silva *et al.* 1997a and b) which are consumed locally. Daniells (2000) made a checklist of the better varieties and their positive and negative characteristics based on yield, stature, susceptibility to wind damage, and diseases. He noted that whether a variety is suitable for dessert or cooking purposes is governed largely by cultural preference. Again some varieties are quick cycling; this may or may not make up for their low-medium bunch weight to give high yields. Daniells (2000) observed that, in Cavendish banana cultivars, even though Dwarf varieties are resistant to wind damage, they tend to be more susceptible to chock throat; a physiological disorder of the bunch. This means that it is not easy to get an all round perfect cultivar that has all the good horticultural characteristics needed. Diseases also play an important part in horticultural trait evaluation in banana, as diseases affects yield and quality. In some cases, diseases reduced yields by up to 100%.

Yield levels achieved in bananas vary greatly depending on soil type, prevailing climate, management level and banana type. In the humid tropics, Stover and Simmonds (1987) attributed the increase in average annual marketable yields of banana of 10 to15 tons ha^{-1} before 1960 to the present day levels of 50 to 60 tons ha^{-1} as due to the adoption of research technology like cultivar choice, horticultural management and plant protection. The removal of healthy leaves from the bananas at flowering resulted in reduction of bunch weight when 4 or less leaves were left (Robins 1996); from the results it was concluded that there should be a minimum of 12 healthy leaves at flowering, and nine at harvest to achieve maximum bunch filling.

CHAPTER 3

MORPHOLOGICAL CHARACTERIZATION OF EAST AFRICAN AAB "APPLE" AND AA "MURARU" BANANAS

3.0. Introduction to Morphotaxonomy

As already mentioned, cultivated bananas are genetically complex, being hybrids of either *Musa acuminata* sub-species exclusively or hybrids of *Musa acuminata* and *Musa balbisiana*. This complexity has made morphological classification difficult. Hybrid cultivars of *Musa acuminata* and *Musa balbisiana* have been classified based on whether the plant has more of *acuminata* morphological characteristics or the reverse (Simmonds 1966). Banana classification based on A/B genome composition and ploidy level (Stover and Simmonds 1962, Simmonds 1966) comes close to the true picture; it serves a useful purpose in reconstructing the pattern of polyploidy in banana (De Langhe 1969) and is what is currently being used in cultivated banana nomenclature. There is, however, more to banana classification than just the proportion of these species found in the genome. As mentioned in Chapter 2, within the AAA genotype alone, the following sub-groups have been named: Cavendish, Gros Michel and Green Red (dessert bananas), and East African highland cooking bananas. Similarly, within the AAB genotype, there is diversity composed of the plantain cooking banana sub-group and various dessert banana sub-groups. Thus, there is no clear connection between the genomic composition of the bananas and their use (De Langhe 1969). Genomic designations are undoubtedly oversimplified. The various *acuminata* and *balbisiana* sub-species combinations

impart to a cultivar its overall phenotype that, when used comprehensively in morphological classification, seems to group them in the various utility groups so that these may be distinguishable from each other based on morphological traits.

Stover and Simmons (1962) distinguish the various genomic groups of bananas based on the 15 morphological features that separate the *acuminata* and *balbisiana*. Similarly, Karamura (1998) was able to morphologically distinguish the various clone sets of AAA East African banana cultivars based on additional characters that were polymorphic among clones of this grouping. Other characterization studies based on morphological traits (Nsabimana *et al.* 2005, Uma *et al.* 2006, Ortiz 1997, Simmonds and Weatherup 1990) have been based on the bearing plant and inflorescence and fruit descriptors. These studies did not include the East Africa AAB "Apple" or AA "Muraru" dessert bananas, however.

It was the purpose of this study 1) to determine morphological characters that distinguish the AAB Apple "Sukari Ndizi" from other AAB "Apple"dessert bananas, 2) to identify morphological features that allow recognition of East African AA "Muraru" dessert bananas, and 3) to evaluate the relationships between East African AAB "Apple" and AA "Muraru" dessert bananas using morphological features.

3.1 Materials and Methods

3.1.1. Study site, accessions and sources

Morphological characterization was conducted at the Kenya Agricultural Research Institutue (KARI) in Kisii, Kenya (latitude 00^0 41'S, longitude 34^0 37'E, altitude of 1800 m above sea level). Forty-three (43) plants were chosen from a

horticultural trial established in October 2005. Data scoring for outgroups were done in the collection established at the same location in 2002. Row spacing between cultivars was 3 m and plants of the same accession were separated within rows by a space of 2 m. The plants were grown under rainfed conditions of an annual average of 1700 mm; no additional irrigation was provided. The average relative humidity was 65%, the mean temperature 21°C, and there was an average of 7 hours of sunshine per day. The soil was an acidic ultisol (pH 5.6- 6.2 at 10-15cm) that was well-drained though poor in nutrients. At planting 20 kg of well-rotted cattle manure plus 200 grams of diammonium phosphate (DAP) was applied per planting hole. Later, the plants were top-dressed with 100 grams of calcium ammonium nitrate (CAN) per mat every 6 months and coinciding with the rains. The orchard was kept weed free by manual weeding of the mats and chemical control (by using Roundup) on the paths.

3.1.2. Choice of morphological characters and character states

Eighty-four characters were chosen from those employed by Bioversity International in consultation with their banana taxonomist, Dr. Deborah Karamura (Table 3.2). The characters selected were easily observable and polymorphic enough to distinguish between various accessions. In addition they could act as morphological descriptors useful for identifying each type. Definitions and explanations of variations in the characters are given below. The scoring of most characters took place at flowering. Others characters were scored at harvest and after fruit ripening. Data were recorded in a field notebook and transferred to Excel spreadsheet data base. A representative plant for each accession was measured for the various characters.

Table 3. 1: Banana accessions included in morphological study.
The study group consisted of the AAB "Apple" and AA "Muraru" bananas. The outgroup consisted of bananas of other groups and were chosen to allow clear separation among the ingroups.

Study groups accessions' local names	Outgroup accessions' local names
AAB "Apple" Mysore	**AAA Cavendish**
Sukari red	Poyo
Wangae red	Pekera
AAB "Apple" Prata	**AAA Gros Michel**
Manyatta	Kampala
Kifutu -Kisii	Pelipita Kisii
Exera	Bogoya
Kifutu -Thika	Pelipita Thika
Soth	Gros Michel
AAB "Apple" Silk	**AAA EAH cooking**
Manjano	Kimuga
Mboki Msukari	Ngombe
Ungoye sweet	
AAB "Apple" "Sukari Ndizi"	**AAB Plantain**
Sweet yellow	Horn plantain
Sweet white	Spambia
Wangae	
Kamaramasenge	
Sukari Ndizi	
Wangae	
Embu	
Kakamega	
AAAB "Apple" tetraploid	**AAAB FHIA hybrid**
GT	Gold finger
AA "Muraru"	**ABB bananas**
Muraru	Ngoja
Muraru Mshare	Kayinja
TT2	
Njuru	
Majimaji	
Mraru Mlalu	
Kamunyilya	
Muraru white green bell	
Muraru red bell	
Makyughu	

Table 3. 2: Morphological characters and character states used to score banana accessions*

PLANT GENERAL APPEARANCE (6.1)

**Leaves habit (6.1.1): erect (1), intermediate (2), drooping (3)

PSEUDOSTEM/SUCKERS (6.2)

Pseudostem height (6.2.1): ≤2 m (1), 2.1 to 2.9 m (2), ≥3 m (3)

Pseudostem aspect (6.2.2): slender (1), normal (2), robust (3)

**Pseudostem color (6.2.3): green yellow (1), medium green, (2) green (3), dark green (4), blue (5)

Pigmentation on the pseudostem (6.2.3.b): none (1), brown/rusty brown (2), black (3)

Predominant underlying color of the pseudostem (6.2.5): watery green (1), light green (2), green (3), pink-purple (4), red purple (5)

Pigmentation of the underlying pseudostem (6.2.6): pink-purple (1), red (2), purple (3)

Wax on leaf sheaths (6.2.8): very little or no visible wax (1), very few wax (2), moderately waxy (3), very waxy (4)

Number of suckers (6.2.9): 0-5 (1), 6-10 (2), 11-20 (3), >20 (4)

PETIOLE/MIDRIB/LEAF (6.3)

Blotches at the petiole base (6.3.1): without pigmentation (1), sparse blotches (2), small blotches (3), large blotches (4), extensive pigmentation (5)

Blotches color (6.3.2): brown (1), dark brown (2), brown black (3), black- purple (4)

Petiole canal leaf III (6.3.3): open with margin spreading (1), wide with erect margins (2), straight with erect margins (3), margins curved inward (4), margins spreading (5)

Petiole margin color (6.3.6): green (1), pink/purple to red (2), purple to blue (3)

Edge of petiole margin (6.3.7): colorless (without a color line along) (1), with a color line along (2)

Petiole margin width [cm] (6.3.8): ≤1 (1), >1 (2)

Leaf blade length [cm] (6.3.9): ≤170 cm (1), 171 to 220 cm (2), 221 to 260 cm (3), ≥ 261 cm (4)

Leaf blade width [cm] (6.3.10): ≤70 cm (1), 71 to 80 cm (2), 81 to 90 cm (3), ≥ 91 cm (4)

Petiole length [cm] (6.3.11): ≤50 cm (1), 51 to 70 cm (2), ≥ 71 cm (3)

Color of leaf upper surface (6.3.12): green yellow (1), medium green, (2) green (3), dark green (4)

Color of leaf lower surface (6.3.14): green yellow (1), medium green, (2) green (3), dark green (4)

Wax on leaves (6.3.16): very little or no visible wax (1), very few wax (2), moderately waxy (3), very waxy (4)

Color of midrib dorsal surface (6.3.20): yellow (1), light green, (2) green (3), pink-purple (4), red-purple (5)

Color of midrib ventral surface (6.3.21): yellow (1), light green, (2) green (3), pink-purple (4),) red-purple (5)

Color of cigar leaf dorsal surface (6.3.22): green (1), purple (2)

***Blotches on leaf of water suckers (6.3.23): without blotches (1), little or narrow blotches (2), large purple blotches (3)

INFLORESCENCE/MALE BUD (6.4)

Peduncle length (cm) (6.4.1): ≤30 cm (1), 31 to 60 cm (2), ≥ 61 cm (3)

Empty nodes on peduncle (6.4.2): none (1), one (2), two or more (3)

Peduncle color (6.4.4): light green, (1) green (2), dark green (3), red or pink-purple (4) with purple, brown or blue blotches (5)

Peduncle hairiness (6.4.5): hairless (1), slightly hairy (2), very hairy, short hairs (3), very hairy, long hairs (4)
Bunch position (6.4.6): hanging vertically (1), slightly angled (2), hanging at 45^0 angle (3), horizontal (4)
Bunch shape (6.4.7): cylindrical, bunch length ≥twice bunch width (1), truncated (2), asymmetrical (3), cylindrical, bunch length <twice bunch width (4)
Bunch appearance (6.4.8): lax (1), compact (2), very compact (3)
Rachis position (6.4.12): falling vertically (1), at an angle (2), with a curve (3), horizontal (4)
***Rachis appearance (6.4.13): bare (1), neutral flowers (2), male flowers/bracts above the male bud (3), neutral/male flowers and presence of withered bracts (4), neutral/male flowers on the whole stalk without persistent bracts (5)
Male bud type (6.4.14): normal (*present*) (1), degenerate before maturity (2), like true-horn plantain (*absent*) (3)
Male bud shape (6.4.15): like a top (1), lanceolate (2), ovoid (3), rounded (4), cordate (5)
Male bud size in length (6.4.16): ≤20 cm (1), 21 to 30 cm (2), ≥ 31 cm (3)

BRACT (6.5)

Bract apex shape (6.5.2): pointed (1), slightly pointed (2), intermediate (3), obtuse (4), obtuse and split (5)
Bract imbrication (6.5.3): old bracts overlap at apex of bud (1), young bracts overlap (2), young bracts greatly overlap (3)
Color of the bract external face (6.5.4): green (1), red-purple (2), purple-brown (3), blue-purple (4), pink-purple (5)
Color of the bract internal face (6.5.5): yellow or green (1), orange red (2), red (3), pink-purple (4), purple-brown (5)
Bract scars on rachis (6.5.8): very prominent (1), prominent (2)
Fading of color on bract base (6.5.9): color discontinous towards the base (1), color homogenous (2)
Male bract shape (6.5.10): x/y<0.28 (*lanceolate*) (1), 0.28>x/y<0.30 (2), x/y >0.30 (*ovate*) (3)
Bract lifting (6.5.11): not lifting from male bud (bracts are persistent) (1), lifting one at a time (2), lifting two or more at a time (3)
Bract behaviour before falling (6.5.12): revolute (rolling) (1), not revolute (not rolling) (2)
Wax on the bract (6.5.13): very little or no visible wax (1), very few wax (2), moderately waxy (3), very waxy (4)

MALE FLOWER (6.6)

Male flower behaviour (6.6.1): falling before the bract (1), falling with the bract (2), falling after the bract (3), neutral and male flowers persistent (4)
***Compound tepal basic color (6.6.2): white (1), cream (2), yellow (3), orange (4), pink/pink-purple (5)
**Compound tepal pigmentation (6.6.3): very few or no visible sign of pigmentation (1), rust-colored (2), presence of pink (3)
Lobe development of compound tepal (6.6.5): little or no visible sign of development (1), developed (2), very developed (3)
Free tepal color (6.6.6): translucent white (1), opaque white (2), tinted with yellow (3), tinted with pink (4)
Free tepal shape (6. 6.7): rectangular (1), oval (2), rounded (3), fan-shaped (4)
Free tepal apex development (6.6.9): little or no visible sign of development (1), developed (2), very developed (3)
Free tepal margins (6.6.9b): not serrated (1), serrated (2)
Free tepal apex shape (6.6.10): threadlike (1), triangular (2), obtuse (3)
Anther exsertion (6.6.11): exserted (1), same level (2), inserted (3)
Anther color (6.6.13): cream (1), yellow (2), brown/rusty brown (3), pink/pink-purple (4), black (5)

Pigmentation on style (6.6.17): without pigmentation (1), purple (2)
Style exsertion (6.6.18): exserted (1), same level (2), inserted (3)
Stigma color (6.6.20): cream (1), yellow (2), pink/pink-purple (3), bright yellow (4), orange (5)
Ovary basic color (6.6.22): white (1), cream (2), yellow (3), green (4)
Ovary pigmentation (6.6.23): very few or no visible pigmentation (1), with red-purple (2)
Dominant color of male flower (6.6.24): white (1), cream (2), yellow (3), pink (4), red-purple (5)
Arrangement of ovules (6.6.26): two-rowed (1), four-rowed (*more or less*) (2)

FRUIT (6.7)

Fruit position (6.7.1): curved inwards stalk (1), parallel to the stalk (2), curved upwards (*oblique, at 45^0 angle upward*) (3), perpendicular to the stalk (4), pendant (5)
Number of fruits in hand (6.7.2): ≤12 (1), 13-16 (2), ≥ 17 (3)
***Fruit length (cm) (6.7.3): ≤15 cm (1), 16-20 cm (2), 21 -25 cm (3), 26-30 (4), ≥31
Fruit shape (6.7.4): straight (or slightly curved) (1), straight in the distal part (2), curved (sharp curve) (3), curved in S shape (double curvature) (4)
General fruit shape (6.7.4b): rounded (1), slender (2), gourd shape (3)
Transverse section of fruit (6.7.5): pronounced ridges (1), slightly ridged (2), rounded (3)
Fruit apex (6.7.6): pointed (1), lengthly pointed (2), blunt-tipped (3), bottle-necked (4), rounded (5)
Remains of flower relicts at fruit apex (6.7.7): without any floral relicts (1), persistent style (2), base of the style prominent (3), persistent and staminode (4)
***Fruit pedicel length [mm] (6.7.8): ≤10 mm (1), 11 to 20mm (2), >21mm (3)
Pedicel surface (6.7.10): hairless (1), hairy (2)
Immature fruit peel color (6.7.12): light green (1), green (2), dark green (3)
**Immature fruit peel appearance (6.7.12b): shiny (1) dull (2), silvery (waxy) (3), very silvery waxy (4)
**Mature fruit peel color (6.7.13): green (1), green-yellow (2), yellow (3), deep yellow (4)
Fruit peel thickness (6.7.14): ≤2 mm (1), ≥3mm (2)
Adherences of the fruit peel (6.7.15): fruit peels easily (1), fruit does not peel easily (2)
Pulp color before maturity (6.7.18): cream (1), ivory (2), yellow (3)
Pulp color at maturity (6.7.19): cream (1), ivory (2), yellow (3), orange (4), cream with brown spots (5)
Flesh texture (6.7.21): firm (1), soft (2)
**Predominant taste (6.7.22): mild, slightly tasty or tasteless (1), sweet (like Cavendish) (2), sugary (like Pisang Mas) (3), sweet and acidic (apple like) (4), sweet and acidic (like AAA cooking) (5), sweet and starchy (like plantain) (6)

*Data form adapted from the International Network for the Improvement of Bananas and Plantains, Musa Germplasm Information System (INIBAP MGIS) data form with the numbering system retained unaltered. Missing characters coded as 999. **Characters useful for distinguishing AA "Muraru", and *** Characters useful for distinguishing AAB "Sukari Ndizi".

3.1.3. Explanation of vegetative characters and variation (6.1-6.3)

The pseudostem is the false stem of the banana plant, consisting of clasping leaf sheaths. The pseudostem height (6.2.1) was measured from ground to level of inflorescence emergence from the pseudostem; this is the point where the penducle (bunch stalk) comes out of the pseudostem before it bends to support the bunch. The pseudostem aspect (6.2.2) was measured as girth at 1meter from ground level.

The background color of the pseudostem (6.2.3) differs in the various genome groups. The clones possessing some *balbisiana*, namely, the AB, AAB, ABB groups, have various shades of light green pseudostems; the AAA dessert bananas (especially Gros Michel) are bright green with brown pigmentation on the pseudostems. Some AAA dessert Cavendish bananas have some shade of blue-purple on their pseudostems, while most East African clones have extensive mottling of black pigment on their pseudostems, almost masking the green color of the stem. The pseudostem color (6.2.3) was assessed using a color chart, found in 'Descriptors for Banana *Musa* spp. (IPGRI, 1999) from a distance of two to three meters from the plant. The color of the blotches (6.2.3b) also varies from brown, dark brown and black depending on the genomic composition. The leaf sheaths of the bananas clasp around the aerial stem, which carries the inflorescence. The outermost sheaths are loosely clasping and can be removed, so two outer sheaths were removed to expose the inner sheath. At the base of the inner sheath, the color was homogeneous and constant. This color was recorded as the underlying color of the pseudostem (6.2.5).

Banana suckers are lateral shoots from the underground corm of the main banana plant that can be used to vegetatively propagate the clone. There are two kinds

of suckers, "sword suckers" and "water suckers." The sword suckers, with narrow blade-like leaves, depend on the mother plant for growth (Stover 1987). When the connection between the sword sucker and the parent plant is cut off and the sword sucker is stressed (for example through weevil attack), the sword sucker cannot draw on the food reserves of the mother plant; blade leaves quickly turn into normal leaves. The broad leaves help to build a photosynthetic capability to supply food for the sucker; these kinds of suckers that have normal leaves are the water suckers. Variation exists among genome groups in the degree of sucker production, and, in this study, the total number of suckers produced from planting to harvest (6.2.9) was scored.

Banana clones differ in whether their water suckers have pigmented blotches on their leaves or not. The blotches are normally seen only in water suckers or young, vigorously growing tissue-cultured plantlets. Scores were made for the presence and intensity of blotches on the leaves (6.3.23).

The petiole is the stalk of the leaf between the sheath and the blade. It varies in length (6.3.11), margin color (6.3.7), and shape of adaxial canal, i.e., whether the margins are spreading, erect, or rolled inward (6.3.3). The 4^{th} leaf below the bunch was cut at the point of attachment on the pseudostem, and the length of the petiole was measured from the adaxial side, starting from the point of attachment on the stem to the base of the lamina. The margin color (6.3.7) and petiole canal feature (6.3.3) were recorded using the 'descriptors for bananas *Musa* spp.' (1999).

Leaves vary in length (6.39), width (6.3.10), and color (6.3.12 and 6.3.14) (Karamura 1998). Leaf length and width were measured using the same leaf from

which petiole measurements were taken. The measurements were taken on level ground immediately after cutting to avoid shrinking. The leaf length was scored from the lamina base to the lamina tip. The width was scored at the widest part of the leaf. The color of the cigar leaf (6.3.22) (the youngest central leaf of a banana plant that is still unfurled) was also recorded. Leaves of the banana crop have various shades of green, and these were also recorded (6.3.12 and 6.3.14).

3.1.4. Reproductive characters and variations (Characters 6.4-6.7, Table 3.2)

The length and hairiness of peduncles (the bunch stalk) vary between clones. The length was recorded from the point of emergence from the pseudostem to the node of the first hand in the bunch (6.4.1). Peduncle hairiness (6.4.5) is a character important in discriminating clones that contain different dosages of the *acuminata* genome (Simmonds, 1966). Groups ABB and AB have a glabrous peduncle; AAB clones are finely hairy; and the AAA triploids, especially the Highland bananas, have coarsely hairy peduncles. The degree of hairiness was scored by feel as a multistate ordered character. Empty nodes on the penducle (6.4.2) and the penducle color (6.4.4) also differ in different bananas and are used for classification.

The rachis (the long inflorescence axis that bears the fruit and the male bud) varied in length, position, color, number of nodes, and the presence or absence of persistent neuter flowers (sterile flowers with both male and female parts that persist on the rachis below the bunch, but do not develop into fruit). The rachis position (6.4.12) and appearance (6.4.13) were scored as multistate unordered characters.

There was high variability in male inflorescence characters among the different genomic groups. In this study, most male inflorescence characters were scored at harvest. As flowering progressed, the volume of the male bud decreased and the shape changed because of the daily opening and falling of the bracts and flowers. The parts of the male inflorescence develop below the last hand of the bunch and continue to the tip of the male bud. All AAB dessert banana clones have male buds, although, among the AAB plantains, the lack of male buds is common. True horn plantains have no male buds; false horn plantains have degenerative male buds; the French plantains have true male buds. The male bud itself varies in many aspects, including bud type (6.4.14), bud shape (6.4.15), and bud size (6.4.16).

Bracts show variation in the way they are arranged in the bud, in their length and width, whether they curl or not after opening, and the color changes inside the bract. The following bract aspects were scored: bract apex shape (6.5.2), bract imbrications (6.5.3), color of bract external and internal face (6.5.4 and 6.5.5, respectively), the fading of color on bract base (6.5.9), bract shape (6.5.10), bract lifting (6.5.11), and wax on the bract (6.5.13). Some authors (Hari 1968) recommend that bract apex shape be rejected as a character in the classification of *Musa acuminata* as it is subject to morphological changes during development. This change was seen during this study; however, the character was useful in clones with a *balbisiana* genome and some Muraru accessions with persistent bracts like Makyungu and TT2. Data were collected at harvest for all accessions to ensure no further changes took place. Bracts were defined as imbricate if the apices of the lower bracts

extended beyond the apices of the upper ones (Hari 1968). Bracts of the male bud were either imbricate or not.

Size variation in male flowers was noted and was useful in discriminating between the cultivars. An advantage of using male flowers was that they do not form fruit and can be used without interfering with the fruit development. Male flowers are smaller than the parthenocarpic "female" flowers, with a very small ovary, slender style and stigma, and anthers that are morphologically well developed. The male flowers abscise from the base of the abortive ovary and are shed whole after being exposed for about a day, whereas the female flowers have no abscission layer at the base of the ovary and are therefore always persistent. Male flower characters (6.6.1 to 6.6.26) were scored one to three months after inflorescence emergence. Characteristics of the compound tepal (6.6.2 to 6.6.5), and free tepal (6.6.6 to 6.6.10) were recorded following the guide of the banana descriptors. The nature of filament and anthers (6.6.11 to 6.6.13), the stigma and style, ovary traits (6.6.17.23) and dominant male flower color were recorded. The arrangement of ovules (6.6.26) was scored 12 to 14 days after the inflorescence emergence and all bracts of the female hands had fallen (Stover 1987); at this stage it was very easy to observe the ovule arrangements and count them in the fruit.

The shape of the mature bunch depends on 2 factors: geotropic reaction and weight (Stover 1987). In many cultivars that belong to the *Musa* section of edible bananas, the fruiting part of the bunch is apparently ageotropic, because it grows horizontally; however heavy bunches will stand obliquely (as seen slightly in Njuru and Kamunyilya of AA "Muraru" subgroup). The male axis may behave differently

from the female, growing vertically downwards (positively geotropic). In still other cultivars, the female part is positively geotropic and the male ageotropic. The response of the fruiting part affects the finger (fruit) orientation with regard to the bunch axis as the fingers are negatively geotropic. Fruit position (6.7.1) was scored as a multistate unordered character.

Bunch and fruit characters were scored at harvest when a bunch first showed a ripened yellow finger (a single individual banana fruit) on the first hand (a cluster of fingers) when the bunch was mature and ready to harvest. Bunch and fruit characters varied greatly because of selection pressures that affect them more than any other part of the plant (Karamura 1998). Fruit size is of commercial importance, although flavor of different clones does not necessarily correspond with the size of the fruit (Karamura 1998). Many consumers prefer bunches with large fruits as, most times, it is not possible to know the flavor or taste of banana fruit just by looking.

Other characters recorded at harvest include peduncles, orientation, length, circumference, shape, and compactness of the bunch. Bunch position (6.4.6), shape (6.4.7), and appearance (6.4.8) are all important discriminating traits in bananas. The degree of compactness of a bunch (6.4.8) is an important character to farmers and buyers. In this study, if one's finger could not fit in between the banana hands, the bunch was considered very compact; if one's hand could be inserted between the fingers, but with a little trouble, the bunch was compact; and if it was easy to put one's hand between any two banana hands, the bunch was lax.

Fruits vary in their arrangement within the bunch, in their position (6.7.1) and number on a hand (6.7.2), length (6.7.3), shape (6.7.4 and 6.7.4b), transverse section

(6.7.5), and shape of apex (6.7.6). The remains of flower relicts at fruit apex (6.7.7), the fruit pedicel length and the surface (6.7.8 and 6.7.10) were recorded.

3.1.5. Nursery stage characters

Nursery stage refers to young plants specifically derived from tissue culture origin. Morphological data were collected at the nursery stage, 2 to 4 months after potting the tissue-cultured plantlets, at the Magoon horticultural facility at the University of Hawaii at Manoa. The objective was to find traits that can help in distinguishing banana accessions at this early stage.

Figure 3. 1: Inner color of pseudostem trait of various banana accessions used in scoring, from top clockwise ABB Kayinja, AAA Cooking, AA "Muraru" and AAA Cavendish

Figure 3. 2: Morphological differences of ABB and AAA genome bananas; petiole base pigmentation, bract color; photo taken 2 weeks after shooting this best time to check ovule arrangement

Figure 3. 3: Morphological traits of various banana accessions used in the scoring of bananas, from top clockwise bunch and fruit traits, male bud and flower traits, petiole canal, and cigar leaf

Figure 3. 4: Ripe fruit traits used in scoring morphology

3.1.5.1. Banana clones source and preparation

A subset of the original 133 banana accessions, consisting of 55 banana accessions (Table 3.3) from AA, AAA, AB, AAB, AAAB, and BB genomic groups, were evaluated. Forty-nine tissue-cultured accessions were obtained from Bioversity International, Leuven, Belgium, in June 2006. Six additional tissue-cultured accessions were obtained from Genetic Technologies Limited, Nairobi, Kenya in July 2006. The plantlets from Kenya were directly planted into pots. The small plantlets from Bioversity International were transcultured for rooting between 26th June 2006 and 28th June 2006 into sterile Magenta® (Magenta Corp., Chicago, IL) boxes containing 30 ml of ½-strength liquid Murashige and Skoog (MS) (1962) minimal organic media. Tissue-cultured banana plantlets were transferred into the media under sterile conditions in a lamina flow cabinet. All of the plants from each test tube/box were transfered into 1 Magenta box. Most of the plantlets had good roots by the 3rd week after transfer. On 17th July 2006 the plantlets were taken for hardening in the greenhouse for a week while still in the Magenta boxes.

Potting of the rooted banana plantlets was done between 24th July 2006 and 26th July 2006 in Pro-mix artificial potting media, containing phosphorous fertilizer. Plantlets were hand irrigated every other day or whenever the media showed signs of drying. After three weeks, plantlets were transferred into larger pots to allow them to grow with minimal restriction. The plantlets grew in the greenhouse under 50% shading for 2 months, and were then removed from the greenhouse to a 10% shade area. Data were collected from these plantlets in October 2006 after evaluation for a subset of 9 traits from Table 3.2. Photographs of some of the accessions showing

nursery traits that were used as morphological descriptors are shown in Figures 3.5 and 3.6. The traits scored were 6.1.2, 6.2.3a and b, 6.2.3, 6.3.6, 6.3.8, 6.3.16, 6.3.21, and 6.3.23; these were polymorphic traits at this stage and could be used for classification. They were scored for each accession and the data were analyzed by a similarity coefficient and cluster analysis using UPGMA on NTSYSpc version 2.2.

3.1.6. Data analysis method

3.1.6.1. Measurement of similarity

In this study Jaccard's and Simple Matching coefficients (Sokal and Michener 1958, Sneath and Sokal 1973, Roger *et al.* 2003) were used to measure similarity between Operational Taxonomic Units (OTUs). The similarity coefficients were then used in the two major approaches in analyzing similarities, i.e., classification and ordination. The data were subjected to both cluster analysis (CA) and principal component analysis (PCA) (Sneath and Sokal 1973, Roger *et al.* 2003). Phenogram constructions were made using the Unweighted Pair Group Arithmetic Mean (UPGMA) algorithm (Sneath and Sokal 1973, Roger *et al.* 2003). Principal component analysis is an ordination technique that produces a visual representation of the relative positions of the OTUs in a space of reduced dimensions, thus summarizing multi-dimentional spatial relationships among OTUs (Chapter 2). Bootstraping, based on 1000 permutations, was done using Phylogeny Analysis Using Parsimony (PAUP) program. The bootstrap values were expressed as percentages and are indicated on the internal branches defining the clusters. Bootstrap values serve as confidence levels for the clusters.

Table 3. 3: Banana accessions used in nursery morphology study

Accession name and ITC code	Source	Subjective grouping
Kifutu	Kisii	AAB Apple
Exera	Kisii	AAB Apple
Ungoye sweet	Kisii	AAB Apple
Muraru Mshare	Kisii	AA Muraru
Njuru	Kisii	AA Muarru
Muraru Mlalu	Kisii	AA Muraru
ITC0083 Igisahira	ITC-Belgium	AAA EAH cooking
ITC0084 Mbwazirume	ITC-Belgium	AAA EAH cooking
ITC0086 Nyamwiihogora	ITC-Belgium	AAA EAH cooking
ITC0141 Kagera	ITC-Belgium	AAA EAH cooking
ITC0154 Mbirabire	ITC-Belgium	AAA EAH cooking
ITC0163 Inyoya	ITC-Belgium	AAA EAH cooking
ITC0168 Imbogo	ITC-Belgium	AAA EAH cooking
ITC0170 Bakurura	ITC-Belgium	AAA EAH cooking
ITC0087 Kayinja (Pisang Awak)	ITC-Belgium	ABB dessert P Awak
ITC0048 Valery	ITC-Belgium	AAA Cavendish
ITC0263 Highgate	ITC-Belgium	AAA Cavendish
ITC0340 Pisang Masak Hijau	ITC-Belgium	AAA Cavendish
ITC 1122 Gros Michel	ITC-Belgium	AAA Gros Michel
ITC0724 Cocos	ITC-Belgium	AAA Gros Michel
ITC0504 FHIA-01 (Gold finger)	ITC-Belgium	AAAB FHIA hybrid
ITC0336 Improved Lady Finger	ITC-Belgium	AAB Apple
ITC0076 Pome	ITC-Belgium	AAB Apple
ITC1441 Pisang Ceylan	ITC-Belgium	AAB Apple
ITC0348 Silk	ITC-Belgium	AAB Apple
ITC0127 Kamaramasenge	ITC-Belgium	AAB Apple
ITC0207 Prata	ITC-Belgium	AAB Apple
ITC 0535 Kluai Roi Wi	ITC-Belgium	AAB Apple
ITC 0582 Lady Finger Nelson	ITC-Belgium	AAB Apple
ITC 0583 Lady Finger S Johnstone	ITC-Belgium	AAB Apple
ITC 0649 Foconah	ITC-Belgium	AAB Apple
ITC 1222 Sport of Silk	ITC-Belgium	AAB Apple
ITC 1275 Yangambi no. 2	ITC-Belgium	AAB Apple
ITC0737 Kingala no. 1	ITC-Belgium	AAB Apple
ITC0743 Figue Famille	ITC-Belgium	AAB Apple
ITC0769 Figue Pome Geante	ITC-Belgium	AAB Apple
ITC0962 Prata Ana	ITC-Belgium	AAB Apple
ITC0987 Auko	ITC-Belgium	AAB Apple
ITC0990 Vunapope	ITC-Belgium	AAB Apple
ITC0459 Ney Poovan	ITC-Belgium	AAB Apple
ITC 1034 Kunnan	ITC-Belgium	AB diploid
ITC0245 Safet Velchi	ITC-Belgium	AB diploid
ITC 0714 Kirun	ITC-Belgium	AA *acuminata*
ITC0269 Niyarma Yik	ITC-Belgium	AA *acuminata*
ITC0249 Calcutta 4	ITC-Belgium	AA *amuninata*
ITC0392 Datil	ITC-Belgium	AA *acuminata*
ITC0428 Higa (*banksii*)	ITC-Belgium	AA *acuminata*
ITC0435 Pisang Mas Ayer	ITC-Belgium	AA *acuminata*
ITC0480 Pisang Buntal	ITC-Belgium	AA *acuminata*
ITC 0672 Pa (Rayong)	ITC-Belgium	AA *acuminata*
ITC 1223 Mshale	ITC-Belgium	AA *acuminata*
ITC 1253 Mjenga "Michel" diploid	ITC-Belgium	AA *acuminata*
ITC 1358 Ngu	ITC-Belgium	AA *acuminata*
ITC0094 Balbisiana	ITC-Belgium	BB wild *balbisiana*
ITC0246 Cameroun	ITC-Belgium	BB wild *balbisiana*

Figure 3. 5: Leaf nursery traits used in morphological evaluation.
From top, left clockwise: AAB Mysore, AAB "Sukari Ndizi," AAB Silk and AAB Prata. Notice purple the blotches on the leaves of Mysore and Prata, absent in "Sukari Ndizi" and Silk.

Figure 3. 6: Stem, petiole nursery traits used in morphological evaluation.
From top left clockwise: AAA Gros Michel, AAB Yangambi no 2, AAB Prata ana and Prata and AAA cooking banana. Notice the various various subtle stem/petiole color variation and pigmentations.

3.3. Results

3.3.1. Overall morphology

The morphological markers were able to separate the various groups and sub-groups. Bootstrap analysis (set at >50% default) supported all groupings in the overall morphology phenogram. The four groupings of AAB "Apple" dessert bananas (Mysore, "Sukari Ndizi," Silk, and Prata) were separated by both cluster analysis and principal component analysis (Figures 3.7 and 3.8). The morphological traits show that AAB "Sukari Ndizi" is closer to AAB Silk than to AAB Prata (Figure 3.7). Members of the AAB Mysore group were more distant (0.590 similarity) to the other AAB "Apple" bananas and plantain was closer (0.605 similarity). The AAB plantain was also separated from the AAB "Apple" and the ABB types. The cluster analysis using morphological traits indicate that the AAB "Apple" dessert banana types are more similar to the AAB plantain types than to the AAA and AA dessert types. Banana accessions with the B genome were clearly separated from those lacking the B genome by cluster analysis and by principal component analysis. However, the bootstrap support for this distinction (Figure 3.7) was low (56%), indicating that it was not robust. Principal component analysis showed the 2nd principal axis differentiates groups with the *balbisiana* genome in the upper half of the scatter diagram and those without in the lower half, but the 2nd principal component axis accounts for only 11% of the total variation. The AA "Muraru" clustered together (0.82 similarity) next to the dessert cultivars AAA Gros Michel and Cavendish with 0.0.640 similarity. The Cavendish clones (Pekera and Poyo) were

separated from the Gros Michel clones (Gros Michel, Bogoya, Kampala, and Pelipita).

3.3.2. Nursery morphology

Figures 3.9 shows a phenogram based on morphological markers at nursery level. The nine nursery traits were not sufficient to distinguish between accessions in all cases but did show some differences. For example, the AA "Muraru" and the AAA Gros Michel were not easy to separate at two to three months after potting when the data was recorded, but it was possible to separate the AAA Cavendish. Nevertheless, at four months, the AAA Gros Michel could be separated from the AA Muraru by the pink flush on the petiole base. Based on nursery morphology, the AAB Lady's Finger (South Johnstone) clustered closely with the AAA/AA dessert bananas, probably because, apart from sharing many traits with these clones, it also had black pigments on the pseudostem, unlike the other AAB clones. The AAA East African cooking bananas were separated from the others based on nursery traits, but they clustered close to the small-statured BB *balbisiana* when subjected to cluster analysis.

The AA "Muraru", the AAA Cavendish, the AAA Gros Michel, the AAB Prata, the AAB Mysore, and the AAB Plantain have purple blotches on their water suckers, but the AAB "Sukari Ndizi" and the AAA East African Highland banana do not have blotches on their water suckers.

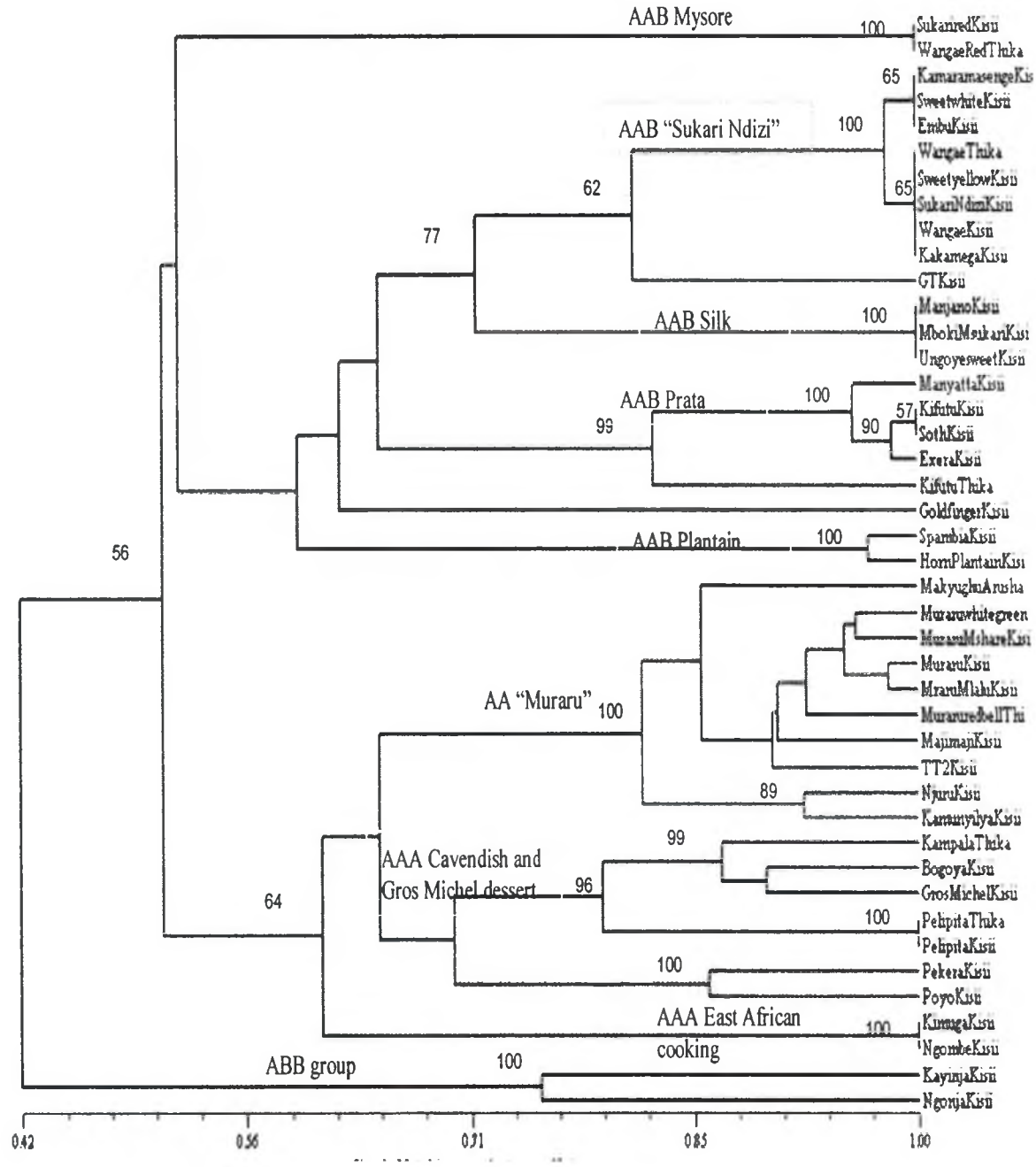

Figure 3. 7: Phenogram from UPGMA between 43 East African accessions of AAB "Apple" and AA "Muraru" and outgroups based on morphological traits.
Cophenetic value =0.889.

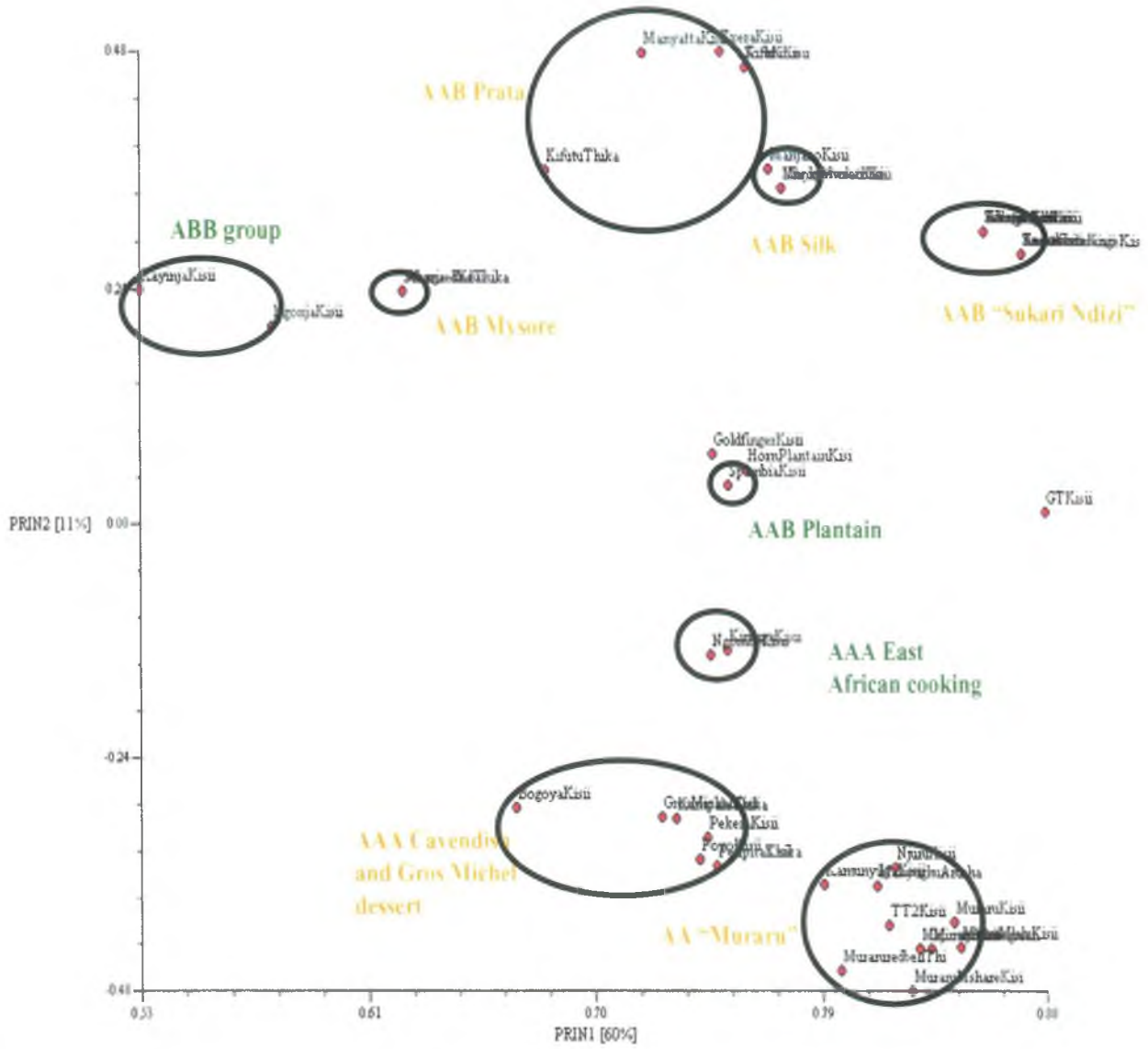

Figure 3. 8: PCA based on morphological traits showing relative positions on the 1st and 2nd principal axes of 43 banana accessions at KARI-Kisii, Kenya

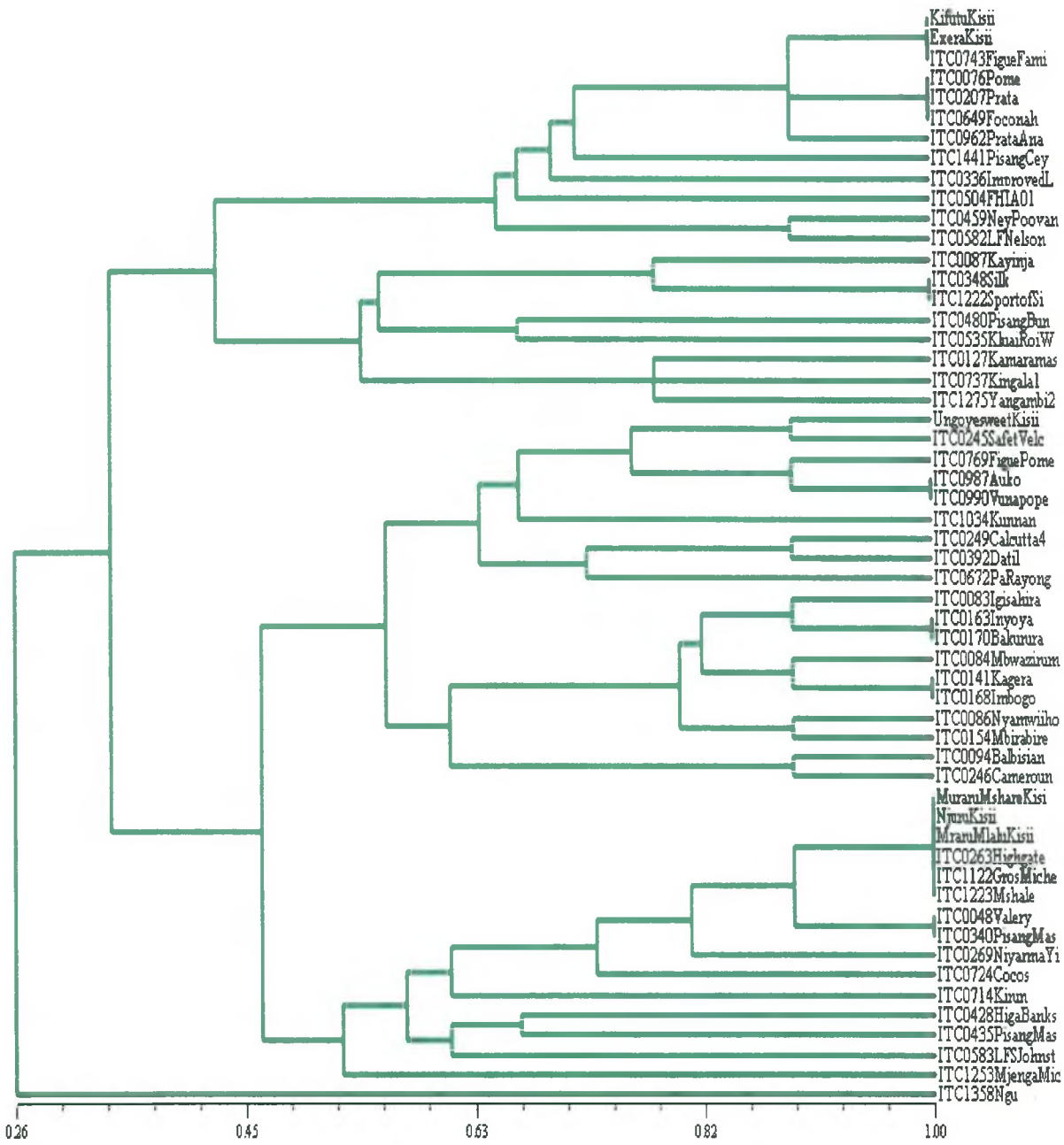

Figure 3. 9: Phenogram from UPGMA among 55 banana accessions based on nursery morphological traits. Cophenetic value = 0.803.

The AAB Prata sub-group had very large purple blotches on their leaves, red-purple midrib and base of the petiole, as well as moderate wax on the base of the petiole. The AAB "Sukari Ndizi" sub-group differed from the AAB Prata because they lack purple blotches on the leaves. However, they had a red-purple midrib and the base of petiole as well as little wax on the base of petiole. The AAB Mysore group differed from the AAB Prata by having little red color on the base of the petiole and a lack of purple blotches. The AAB Mysore had a red-purple leaf background.

The AAB plantains such as ITC0990 Vunapope and ITC0987Auko, and the AB genome clones, namely ITC1034 Kunnan and ITC0245 Safet Velchi had green midribs, a little red-purple color at the base of petiole, and lacked purple blotches on leaves. The AAB ITC1275 Yangambi number 2 and ITC0737 Kingala1 were tall and vigorous. These two had red midribs, lacked purple blotches on the leaves, but had bright red lace at the edge of the petiole base.

The AAA dessert banana group had purple blotches and red lamina edge with little or no red base of the petiole. They also had little wax on the sheaths and a green pseudostem with scanty black pigmentation. They also had some red mid-rib at the sheath ends. The AA "Muraru" shared traits from both the AAA Cavendish and the Gros Michel dessert bananas groups having purple blotches on leaves, little wax, a green midrib, with scant red midrib at the lamina end.

The AAA East African highland bananas had little wax, no purple blotches on the leaves, a green midrib and a green petiole base making them easy to separate from the rest. They also had distinct black pigmentation on the pseudostem (Figure 3.6).

3.4. Discussion

Multivariate analyses indicate that the AAB dessert banana types are morphologically more similar to AAB plantain types than to the AAA and AA dessert types. The morphological phenogram gives a phenetic depiction of the relationships between the groups. Multivariate analyses based on 84 mophological traits distinguish the same clusters as the molecular analyses based on microsatellite markers. The use of many characters apparently overcomes some of the morphological limitations.

At the nursery level, the AA "Muraru" shared traits from both AAA Cavendish and Gros Michel groups, making the three dessert banana groups difficult to tell apart. However, it was still possible with more experience in observation to distinguish the three groups of bananas at the nursery level.

Unlike clones containing B genomes that were of small stature with small slender leaves, the AAA East African cooking bananas are tall and robust with large leaves. They also had wax on the base of petiole, but no pigmentation. The similarity of appearance of BB *balbisiana* and AAA East African highland at the nursery stage was probably influenced by of lack of purple pigmentation on the leaves. Some of the traits used were very relative and not easy to categorize, e.g., the amount of purple blotches on the leaves. ITC0249 Calcutta 4 an AA genome clone, was easy to separate from others as it had shiny green leaves, and a green pseudostem with brown pigmentation. It is important to note that the plants have to be at their optimal growth, having adequate water and favorable temperature in order to score character states reliablly, conditions normally found in plant nurseries.

The loss of the ability to sort similar phenotypes into different AAB groupings when fewer characters are analyzed is evident in the nursery stage evaluation, at which point of development, only nine polymorphic vegetative traits were convenient to measure. While certain vegetatively distinct groupings, such as "Sukari Ndizi" or AAA East African highland cooking bananas, clustered neatly together in the analysis, others are more dispersed (Figure 3.9). The AAB Prata makes up the bulk of a cluster that also includes the Mysore clones Pisang Ceylan and a few other AB, AAB, and AAAB clones. The Silks are more dispersed, occurring in two clusters sharing only 0.3 similarity (Figure 3.9).

3.5. Identification of key characters that describe the East African AAB "Sukari Ndizi" and AA "Muraru" dessert bananas

3.5.1. The East African AAB "Sukari Ndizi"

Watch for the following:

- cream compound tepal basic color without pigmentation
- no blotches on the leaves of water suckers
- fruits $\leq$ 15cm in length with short pedicel
- green yellow pseudostem with brown/ rusty brown pigmentation
- green cigar leaf

3.5.1.1. Describing the East African AAB Sukari Ndizi"

The East African AAB "Sukari Ndizi" banana is a distinct taxon from the AAB Prata, the AAB Mysore and the AAB Silk taxa within the AAB "Apple" dessert banana taxon. The "Sukari Ndizi" accessions clustered together as a distinct taxon of the AAB small-fruited dessert bananas using both microsatellites and morphological markers. The AAB "Sukari Ndizi" taxon can be described as having the following characteristics:

They are small-fruited triploid natural hybrids of *M. acuminata* and *M. balbisiana* Colla having a genomic composition of these two at a proportion of 2:1, respectively. The ploidy level and genomic composition have been confirmed with flow cytometry as a triploid. They have an intermediate leaf habit that may be mistaken for an erect habit especially when they are grown in very fertile soils where they may grow tall (>3 metres) and slender. They have a cream compound tepal basic

color without pigmentation, in clear contrast with the pink/ pink-purple pigmentation of the Mysore, the Silk, the Prata taxa, and the Kisubi. The "Sukari Ndizi" have short pedicel and the ovules are arranged in 2 rows characteristic of *acuminata*. Unlike the Mysore and the Prata that have water suckers that have purple blotches "Sukari Ndizi" has no blotches on the leaves of water suckers. They are less robust than Prata and their pseudostem is generally straighter than the Silk (which have a slightly arched pseudostem). Although all four AAB "Apple" banana taxa, the Prata, Mysore, Silk and "Sukari Ndizi," have a bare rachis at the time of fruit maturity, the "Sukari Ndizi" rachis is always bare even just a few weeks after flowering. In contrast to the Prata that have longer >20cm waxy silvery fruits, the "Sukari Ndizi" has smaller fruits ≤ 15cm in length with short pedicels. Plants have slender to normal pseudostem that are of medium to tall height depending on the stage of crop growth and also on the fertility and rainfall of the growing conditions. Pseudostem color is green yellow with brown rusty brown pigmentation. The petiole margins of the "Sukari Ndizi" are curved inwards, a characteristic of *balbisiana*. The "Sukari Ndizi" can be distinguished from the Mysore because the Mysore has medium long fruits ≥16≤18 that are slightly waxy and a darker green pseudostem with black pigmentation and purple midrib, and a purple cigar leaf. The "Sukari Ndizi" has green cigar leaf and a light green midrib.

The "Sukari Ndizi" has an average bunch weight of 8kg with an average of 8 hands per bunch, and a mean of 100 fruits per bunch. Each hand has an average of 12 fruits each weighing 80grams and the average weight of the hand is 1kg. The fruit is green while mature but unripe and turns yellow when ripe. Pulp color is cream while

mature green and yellow when ripe. Unlike fruits of most of the Silk sub-group that have pulp that is dry with hard brownish parts in the flesh, "Sukari Ndizi" has firm textured spotless uniform cream pulp flesh. Although the "Sukari Ndizi" fruits appear to resemble those of the Mysore at maturity and when fully ripe, the fruit of the "Sukari Ndizi" have thicker peels and a more firm texture with an acidic taste, unlike the Mysore fruits that have a sweeter taste. Silk banana fruits are non-acidic and sweet.

3.5.2. The East African AA "Muraru"

Watch for the following:

- erect leaf habit, edge of petiole margin with pink-purple color line
- fruits lengthily pointed shiny green
- fruits $\geq$ 20 cm long and green to green-yellow when ripe
- normal pseudostem aspect with medium green color having brown-black pigmentation
- bract external surface is purple brown and no wax
- blotches on the leaves of water suckers

3.5.2.1. Describing the East African AA "Muraru"

The East African AA "Muraru" dessert banana is a distinct taxon of AA dessert banana, separate from the AA Sucrier, AA banksii and other AA accessions studied. The microsatellite and morphological markers were able to separate them as a distinct taxon of the *M. acuminata*. Their ploidy level has been confirmed with

flow cytometry as diploid. This group of bananas can be described as having the following characteristics:

They are a medium- to long fruited-diploid of *M. acuminata* Colla only. Unlike most diploid AA bananas that have small fruits, the "Muraru" AA do have fruits that may surpass those of some Cavendish and Gros Michel accessions in size. They have an erect leaf habit that shows their ploidy level, and they grow tall (>3 meters), with a normal pseudostem. They have a cream colored compound tepal with brown pigmentation, and a green ovary basic color. The fruits have short pedicels. Unlike the East African highland banana, they have purple blotches on the leaves of water suckers, just like the Cavendish and Gros Michel. For most "Muraru," the rachis has few neutral flowers, but some have persistent withered bracts on the rachis.

The "Muraru" fruits are long and slender (>20 cm) relative to other diploid bananas, and have gradually tapering points. The mature unripe fruits are green and shiny and they remain green yellow to yellow when ripe, unlike the Gros Michel and the Cavendish dessert bananas which become golden yellow. The pseudostem color is medium green with a little rusty brown to black pigmentation. A bunch of the "Muraru" weighs, on average, 16kg, with a mean 8 hands that weigh 2kg. There is an average of 16 fruits per hand, each weighing 125grams. The pulp color is cream while mature green, and ivory to cream when mature ripe. Unlike the fruits of the Pisang Mas AA that is sugary, the "Muraru" has a mild, slightly tasty or bland predominant taste. The fruit of the "Muraru" have thicker peels and a firmer texture than the Pisang Mas.

CHAPTER 4

ANALYSIS OF GENETIC DIVERSITY AND RELATIONSHIPS IN EAST AFRICAN DESSERT BANANA ACCESSIONS OF *MUSA* AAB "APPLE" AND AA "MURARU" GROUPS USING NUCLEAR MICROSATELLITE MARKERS

4.0. Introduction to use of microsatellites in banana characterization

The analysis of DNA has become increasingly valuable as a tool for the characterization of varieties, evolutionary studies, population genetics, and systematic relationships in many organisms. The potential advantages of DNA-level analyses include the fact that the DNA can be prepared from small amounts of tissue at any stage of life cycle, and that it is relatively stable. Molecular methods that have been employed in the characterization and evaluation of genetic diversity in *Musa* species are described in Chapter 2. These include Restriction Fragment Length Polymorphisms (RFLPs) (Carreel *et al.* 2002); Amplified Fragment Length Polymorphism (AFLP) (Ude *et al.* 2002, 2003); Random Amplified Polymorphic DNA (RAPD) (Pillay *et al.* 2000, 2001; Onguso *et al.* 2004; Kahangi *et al.* 2002), and Simple Sequence Repeat (SSR) or microsatellites (Grapin *et al.* 1998, Creste *et al.* 2003, 2004). The present study is the first to look at East African dessert banana cultivars using SSR markers.

In this study, both nuclear and chloroplast SSR markers were used to analyze the East Africa AAB "Apple" and AA "Muraru" banana clones. Chapter 4 deals with the use of nuclear microsatellite markers in the study of East Africa AAB "Apple"

and AA "Muraru" banana clones, and Chapter 5 will cover the use of chloroplast microsatellite markers in the study of these same clones.

4.1 Specific objectives of the microsatellite study

The following objectives are specific to this study;

1. To screen the East African AAB "Apple" and AA "Muraru" accessions using SSR markers in order to provide an accurate classification of the germplasm, relative to standard clones from a world collection, on which agronomy and breeding work can be based; and
2. To define putative groups and sub-groups within the East African AAB "Apple" and AA "Muraru" dessert bananas

4.2. Materials and methods

4.2.1. Criteria for site selection

Site selection of the areas from which to collect banana samples was based on background knowledge of the presence of the target study material in the areas. Samples were collected from four East African countries, namely, Uganda (1 site), Kenya (2 sites), Tanzania (2 sites), and Rwanda (1 site), shown in Figure 4.1. To ensure an efficient, yet inclusive sampling, the sites selected in each of these four countries were judged to have the greatest diversity for either AAB "Apple" or AA "Muraru" groups of bananas.

4.2.1.1 Banana accessions studied and sample collection

This study concentrated on accessions of the East African AAB "Apple" and AA "Muraru" dessert banana groups collected from existing banana collections at these locations:

1. Regional Research Centre of the Kenya Agricultural Research Institute (KARI) at Kisii, Kenya;
2. National Horticultual Research Centre of KARI at Thika, Kenya;
3. *Institut des Sciences Agronomiques du* Rwanda, Rubona, Butare, Rwanda;
4. National Agricultural Research Station collections at Tengeru and Zanzibar, Tanzania; and
5. Local farmers' fields in the region around Entebbe, Uganda.

All banana accessions used in the study are listed in Tables 4.1, 4.2, 4.3 and 4.4. For the molecular analysis, leaf samples were collected from a total of 133 accessions consisting of 64 from East Africa, 20 Polynesian accessions, and 49 reference accessions from Bioversity International (Leuven, Belgium). Accessions that were not of AAB "Apple" and AA "Muraru" types were used as outgroups. The reference banana accessions from Bioversity International, Belgium were accessed as tissue cultured plantlets. Samples of fresh leaves were kept on ice at 4°C during transportation to the laboratory.

Table 4. 1: Banana accessions sourced from Kenya

Accession local name	Collection	Subjective grouping
Muraru green bell	Thika	Muraru
Muraru red bell	Thika	Muraru
Kampala	Thika	Gros Michel
Pelipita	Thika	Gros Michel
Kifutu	Thika	Apple
Wangae red	Thika	Apple
Wangae	Thika	Apple
Sukari red	Kisii	Apple
Kamaramasenge	Kisii	Apple
Sweet white	Kisii	Apple
Sweet yellow	Kisii	Apple
Kifutu	Kisii	Apple
Exera	Kisii	Apple
Soth	Kisii	Apple
Manyatta	Kisii	Apple
Sukari Ndizi	Kisii	Apple
Wangae	Kisii	Apple
Embu	Kisii	Apple
Kakamega	Kisii	Apple
Manjano	Kisii	Apple
Mboki Msukari	Kisii	Apple
Ungoye sweet	Kisii	Apple
GT	Kisii	Apple
Gold finger	Kisii	FHIA01* hybrid
Muraru	Kisii	Muraru
Muraru (Mshare)	Kisii	Muraru
TT2	Kisii	Muraru
Njuru	Kisii	Muraru
Majimaji	Kisii	Muraru
Mraru (Mlalu)	Kisii	Muraru
Kamunyilya	Kisii	Muraru
Kayinja	Kisii	Dessert / beer
Ngoja	Kisii	Cooking
Spambia	Kisii	Plantain
Horn plantain	Kisii	Plantain
Kimuga	Kisii	EAH** cooking
Ngombe	Kisii	EAH cooking
Pekera	Kisii	Cavendish
Poyo	Kisii	Cavendish
Bogoya	Kisii	Gros Michel
Pelipita	Kisii	Gros Michel
Gros Michel	Kisii	Gros Michel

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Figure 4. 1: Map of East African countries showing collection sites (green stars)

Table 4. 2: Banana accessions sourced from other East African countries

Accession local name	Country source	Subjective grouping
Mlelembo	Arusha, Tanzania	Muraru
Ijighu Inkundu	Arusha, Tanzania	Muraru
Huti white bell	Arusha, Tanzania	Muraru
Kahuti	Arusha, Tanzania	Muraru
Makyughu	Arusha, Tanzania	Muraru
Huti green bell	Arusha, Tanzania	Muraru
Kipaka	Zanzibar, Tanzania	AA diploid
Kisukari	Zanzibar, Tanzania	Apple
Pukusa	Zanzibar, Tanzania	Apple
Kijakazi	Zanzibar, Tanzania	Apple
Paji	Zanzibar, Tanzania	Apple
Kikonde	Zanzibar, Tanzania	Apple
Gisubi	Butare, Rwanda	Apple
Kamaramasenge	Butare, Rwanda	Apple
Poomes	Butare, Rwanda	Apple
Mutsari (Silk)	Butare, Rwanda	Apple
Prata	Butare, Rwanda	Apple
Guindi	Butare, Rwanda	Apple
Kisubi (2)	Entebbe, Uganda	Apple
Kisubi (1)	Entebbe, Uganda	Apple
Sukari Ndiizi (1)	Entebbe, Uganda	Apple
Sukari Ndiizi (2)	Entebbe, Uganda	Apple

Table 4. 3: Banana accessions sourced from Polynesia

Accession local name	Country source	Subjective grouping
Dwarf Brazilian	Oahu, Hawaii	Apple
Tall brazilian	Maui, Hawaii	Apple
Raja puri	Maui, Hawaii	Apple
Kahanu Manzano	Maui, Hawaii	Apple
Manzano	Maui ,Hawaii	Apple
Bluggoe Largo	Maui, Hawaii	ABB cooking
Dippig	Maui, Hawaii	Philippine Saba
Musa balbisiana	Oahu, Hawaii	BB *balbisiana*
Musa acuminata	Oahu, Hawaii	AA *acuminata*
Musa acuminata ssp zebrina	Oahu, Hawaii	AA *acuminata*
Sucrier	Maui, Hawaii	AA *acuminata*
Maia hapai	Maui, Hawaii	AA *acuminata*
Maoli MM	Oahu, Hawaii	Pacific plantain
Maoli HP	Oahu, Hawaii	Pacific plantain
Popo'ulu Hua moa	Maui, Hawaii	Pacific plantain
Maoli- popo'ulu eke-'ula	Maui, Hawaii	Pacific plantain
Iholena Upehupehu	Maui, Hawaii	Pacific plantain
Iholela lele	Maui, Hawaii	Pacific plantain
Popo'ulu ka'io	Maui, Hawaii	Pacific plantain
Puerto Rican dwarf plantain	Maui, Hawaii	plantain

Table 4. 4: Banana accessions used as reference material from Bioversity International, International Transit Centre (ITC), Leuven, Belgium

Accession name and ITC code	**Subjective grouping**
ITC0083 Igisahira	EAH* cooking
ITC0084 Mbwazirume	EAH cooking
ITC0086 Nyamwiihogora	EAH cooking
ITC0141 Kagera	EAH cooking
ITC0154 Mbirabire	EAH cooking
ITC0163 Inyoya	EAH cooking
ITC0168 Imbogo	EAH cooking
ITC0170 Bakurura	EAH cooking
ITC0087 Kayinja (Pisang Awak)	P Awak
ITC0048 Valery	Cavendish
ITC0263 Highgate	Cavendish
ITC0340 Pisang Masak Hijau	Cavendish
ITC 1122 Gros Michel	Gros Michel
ITC0724 Cocos	Gros Michel
ITC0504 FHIA-01 (Gold finger)	FHIA** hybrid
ITC0336 Improved Lady Finger	Apple
ITC0076 Pome	Apple
ITC1441 Pisang Ceylan	Apple
ITC0348 Silk	Apple
ITC0127 Kamaramasenge	Apple
ITC0207 Prata	Apple
ITC 0535 Kluai Roi Wi	Apple
ITC 0582 Lady Finger Nelson	Apple
ITC 0583 Lady Finger S Johnstone	Apple
ITC 0649 Foconah	Apple
ITC 1222 Sport of Silk	Apple
ITC 1275 Yangambi no. 2	Apple
ITC0737 Kingala no. 1	Apple
ITC0743 Figue Famille	Apple
ITC0769 Figue Pome Geante	Apple
ITC0962 Prata Ana	Apple
ITC0987 Auko	Apple
ITC0990 Vunapope	Apple
ITC0459 Ney Poovan	Apple
ITC 1034 Kunnan	AB diploid
ITC0245 Safet Velchi	AB diploid
ITC0269 Niyarma Yik	AA *acuminata*
ITC0249 Calcutta 4	AA *acuninata*
ITC0392 Datil	AA *acuminata*
ITC0428 Higa (*banksii*)	AA *acuminata*
ITC0435 Pisang Mas Ayer	AA *acuminata*
ITC0480 Pisang Buntal	AA *acuminata*
ITC 0672 Pa (Rayong)	AA *acuminata*
ITC 0714 Kirun	AA *acuminata*
ITC 1223 Mshale	AA *acuminata*
ITC 1253 Mjenga "Michel" diploid	AA *acuminata*
ITC 1358 Ngu	AA *acuminata*
ITC0094 Balbisiana	BB *balbisiana*
ITC0246 Cameroun	BB *balbisiana*

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4.2.2. DNA isolation

DNA was extracted from accessions at the International Institute for Tropical Agriculture (IITA) laboratory based at the International Livestock Research Institute (ILRI), Nairobi, and at the Department of Tropical Plant and Soil Sciences, University of Hawai'i at Manoa, Honolulu, Hawai'i. Approximately 10 grams of the youngest unfurled leaf (cigar leaf) from each specimen was initially ground in liquid nitrogen with a mortar and pestle. DNA extraction followed, using the protocol for DNeasy Plant Mini Kit (Qiagen Sciences, Maryland). The DNA in the extract was precipitated by adding one-tenth volume of 3 M sodium acetate (NaOAc, pH 6.8), followed by two volumes of cold ethanol and centrifugation at 6,000 rpm for 5 min to enable transportation from Kenya to University of Hawai'i at Manoa, Honolulu, Hawai'i.

For analysis, each DNA sample was pelleted by centrifugation, washed with 70% ethanol, air-dried briefly, and re-suspended in 200 µl of Tris-EDTA (TE) buffer. DNA concentrations were quantified with a Nano drop, model ND-1000 V 3.30 spectrophotometer. The quality of the DNA obtained was also checked with the same instrument to ensure that the A260nm/A280nm values ranged between 1.4 and 2.2. A 5µl sample of each DNA was run in a 1% agarose gel stained in 5 µg/ml of ethidium bromide solution and compared visually with 2-log ladder of DNA standards of a known concentration under UV illumination. For PCR amplifications, aliquots of each DNA sample were further diluted in filtered sterilized de-ionized distilled water to achieve a concentration of 10 ng per µl. These were stored in a refrigerator (4 °C)

for use in subsequent assays, while the remainder of the DNA material was stored at −20 °C.

4.2.3. Banana nuclear microsatellite markers

Microsatellite markers had been previously identified by other researchers (Crouch *et al.* 1998, Lagoda *et al.* 1998, Creste *et al.* 2003 and 2004) for bananas. These microsatellite markers used previously were screened in an effort to identify suitable primer pairs to use in the evaluation of the East African AAB "Apple" and AA "Muraru" dessert bananas. Thirteen primer pairs, Ma-1-16, Ma-1-17, Ma-1-27, Ma-3-48, Ma-3-60, Ma-3-81, Ma-3-90, Ma-3-92, Ma-3-103, Ma-3-104, Ma-3-109, Ma-3-139, and AGMI 24/25, were screened using 12 banana accessions of AA, BB, ABB, AAB, and AAA genome groups. Optimization of the PCR protocol was done based on published protocols (Crouch *et al.* 1998). Three primers Ma-3-81, Ma-3-92, and AGMI 24/25 did not give usable results and were not used.

4.2.3.1. Polymerase chain reaction (PCR), primer pairs and agarose gel electrophoresis

Polymerase chain reactions were done using an Applied Biosystems Gene Amp® PCR system 2700 thermal cycler programmed to perform 32 cycles. The associated PCR parameters were initially done as described in Crouch *et al.* (1998), but later modified (as described below) to optimize the results. Each reaction was carried out in a total volume of 25 µl containing 10 ng of genomic DNA, 12.5 µl of Amplitaq Gold master mix (Applied Biosystems), 1.5 µl $MgCl_2$, 9.5 µl of filtered distilled de-ionized water and 0.25 µl of each primer.

The following cycling protocol was used: An initial denaturation step at 95^{0}C for 5minutes (1X), followed by 32 cycles of denaturation at 94^{0}C for 1 minute, annealing at 58^{0}C (or 62^{0}C for Ma1-16 and 27) for 1 minute, and extension for 90 seconds at 72^{0}C. A final extension was carried on at 72^{0}C for 7 minutes.

A total of 8 fluorophore-tagged SSR primer pairs (Crouch *et al.* 1998), shown in Table 4.5, were purchased from Applied Biosystems (ABI) and used.

For gel electrophoresis, a 10µl fraction of each amplification reaction was separated at 100v for approximately 2 hours using 2% agarose gels (0.5xTBE buffer and containing ethidium bromide). Gel images were photographed using UV illumination to confirm the presence of an amplification product and to document any polymorphisms.

Table 4. 5: SSR primer number, annealing temperature (Tm) used in this study, fluorophore tag, and microsatellite repeat

Primer number	**Tm (^{0}C)**	**Fluorophore tag**	**Repeat***
Ma-1-16	62	ROX	(GA)10
Ma-1-27	62	FAM	(GA)9
Ma-3-48	58	ROX	(GA)15
Ma-3-60	58	FAM	(GA)14
Ma-3-90	58	HEX	(CT)11
Ma-3-103	58	ROX	(CT)10
Ma-3-104	58	HEX	(GA)10
Ma-3-139	62	FAM	(GA)14

*The microsatellite repeat source: Crouch *et al.* 1998, Lagoda *et al.* 1998, Creste *et al.* 2003.

4.2.3.2. Capillary gel electrophoresis

A 1µl aliquot of the amplified product of each sample was used for genotyping by capillary gel electrophoresis at the Centre for Genomics, Proteomics, and Bioinformatics Research (CGPBRI) at the University of Hawai'i at Manoa. The data obtained were analyzed using the 'Gene Marker,' version 1.51, software program (SoftGenetics, Inc.) Allele calls were identified and, for each accession, presence was given a score of 1, while a score of 0 was given for absence of an allele.

4.2.3.3. Data management

Microsatellite marker alleles, from each of the SSR markers acquired from data analyzed by 'Genemarker' software program, were treated as character states and were entered into an Excel spreadsheet. A character is a well-defined feature of an organism that distinguishes an individual or groups from another and that, in a taxonomic unit, can assume one of two or more mutually exclusive character states. Discrete characters can be assigned two or more states as the case of SSR alleles found at a specific genomic region. When a character can have only two states, it is referred to as binary. When three or more states are possible, the character is referred to as multistate.

4.2.3.4. Data analyes from microsatellite markers

Molecular data provides many binary characters that are useful in phylogenetic studies. In this study, the presence (1) or absence (0) of specific alleles constituted

binary data that was entered in the Excel spreadsheet and then used to build phylogenetic trees and for principal component analysis.

A total of 80 distinct alleles were identified from the 8 microsatellite markers (Table 4.5) and used as SSR characters or descriptors. Alleles were scored for all 8 markers in all 133 accessions. Data were entered in Excel spreadsheet as discrete characters. Each accession that differed by at least one allele was classified as distinct. Using these data, the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc), version 2.2 (Exeter Software, Setauket, NY, USA) determined the genetic similarities between all accessions based on simple matching and Jaccard's correlation coefficients. These data were subjected to cluster analysis (CA) and principal component analysis (PCA).

The large number of samples made it difficult to display in a single page the phenogram in a way that allowed individual accessions to be identified; consequently, in the subsequent analyses, the outgroups were reduced to retain only selected outgroups, namely: Gros Michel AAA, Cavendish AAA, East African Highland cooking banana AAA, and Plantain AAB and ABB types for a total of 103 cultivated accessions. The AA plus AAA groups, BB plus ABB and AB groups, AAB plus AAAB and AB groups, AAA group only and AA group only were subjected to further cluster analysis. Relationships among accessions were evaluated with a phenetic cluster analysis, using the unweighted pair-grouping with arithmetic mean (UPGMA) clustering and plotted in a phenogram using NTSYSpc, version 2.2 (Rohlf 2006). Principal component analysis (PCA) was performed, and the first four

principal axes were used to plot scatter diagrams that show the relationships among the various accessions.

To test the accuracy of the phylogenetic trees, the cophenetic correlations method developed by Sokal and Rohlf (1962) was used. A two way Mantel statistic test of 1000 permutations was performed to get a cophenetic value.

4.3. Results

4.3.1 Allele report

Figures 4.2, 4.3, 4.4, and 4.5 are examples of the nuclear SSR allele reports resulting from genotyping by capillary gel electrophoresis. The nuclear allele reports show as few as one to as many as four alleles at any one locus because cultivated bananas can be diploid, triploid, or tetraploid. To visualize the allele reports, the results obtained from the genotyping were analyzed using Genemarker® 2004, version 1.51 (Soft Genetics LLC®, State College, PA).

Cophenetic correlation coefficients (r_{cs}) between the similarity values implied by the phenogram and those of the original similarity matrix were calculated for all the phenograms (Sneath and Sokal 1973). All of the cophenetic values (r) obtained for the phenograms were above 0.9 except for one phenogram that had a value of 0.892. There was satisfactory agreement of the phenograms with their similarity matrix (Sneath and Sokal 1973).

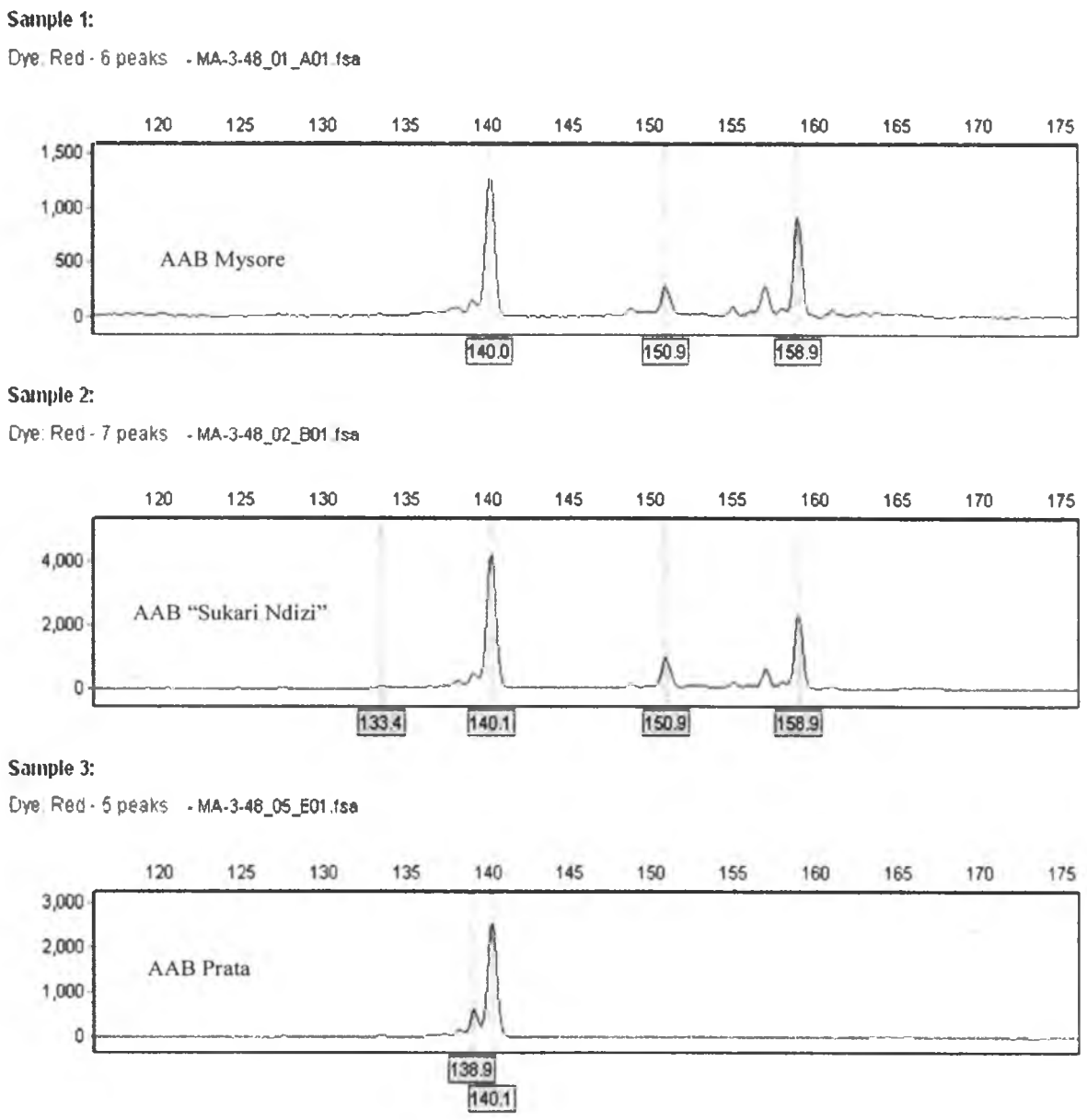


Figure 4. 2: Allele report of SSR primer ma3-48 for the three banana accessions named. Each x-axis shows fragment size in basepair and y-axis show height of fragment peak. AAB Mysore and AAB "Sukari Ndizi" have three alleles each and AAB Prata one allele at this locus.

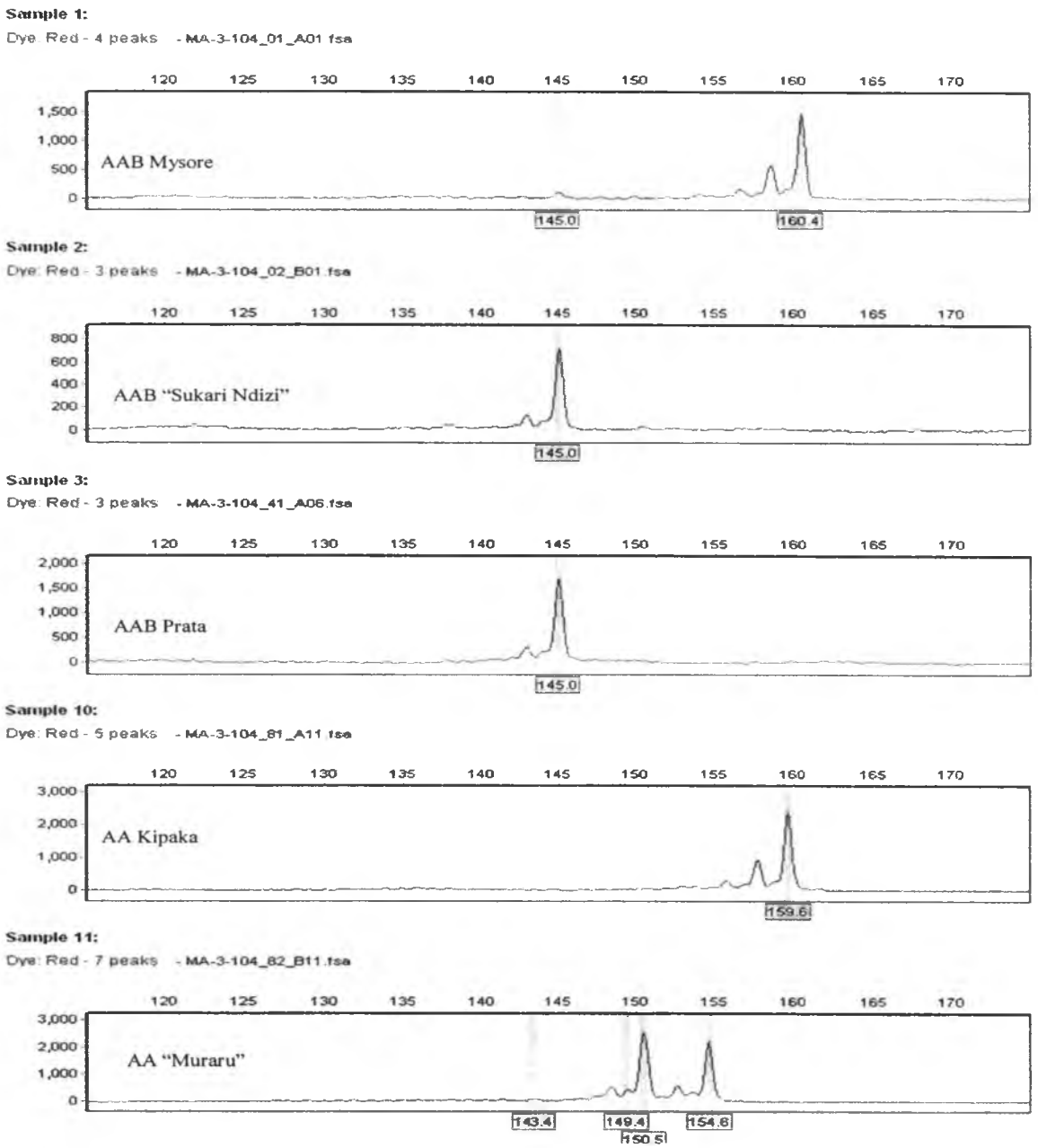


Figure 4. 3: Allele reports for SSR primer ma3-104 for five representative banana accessions. Each x-axis shows fragment size in basepair and y-axis show height of fragment peak. AAB Mysore and AAB "Sukari Ndizi," AA Kipaka and AAB Prata have one allele each and AA "Muraru" has two alleles at this locus.

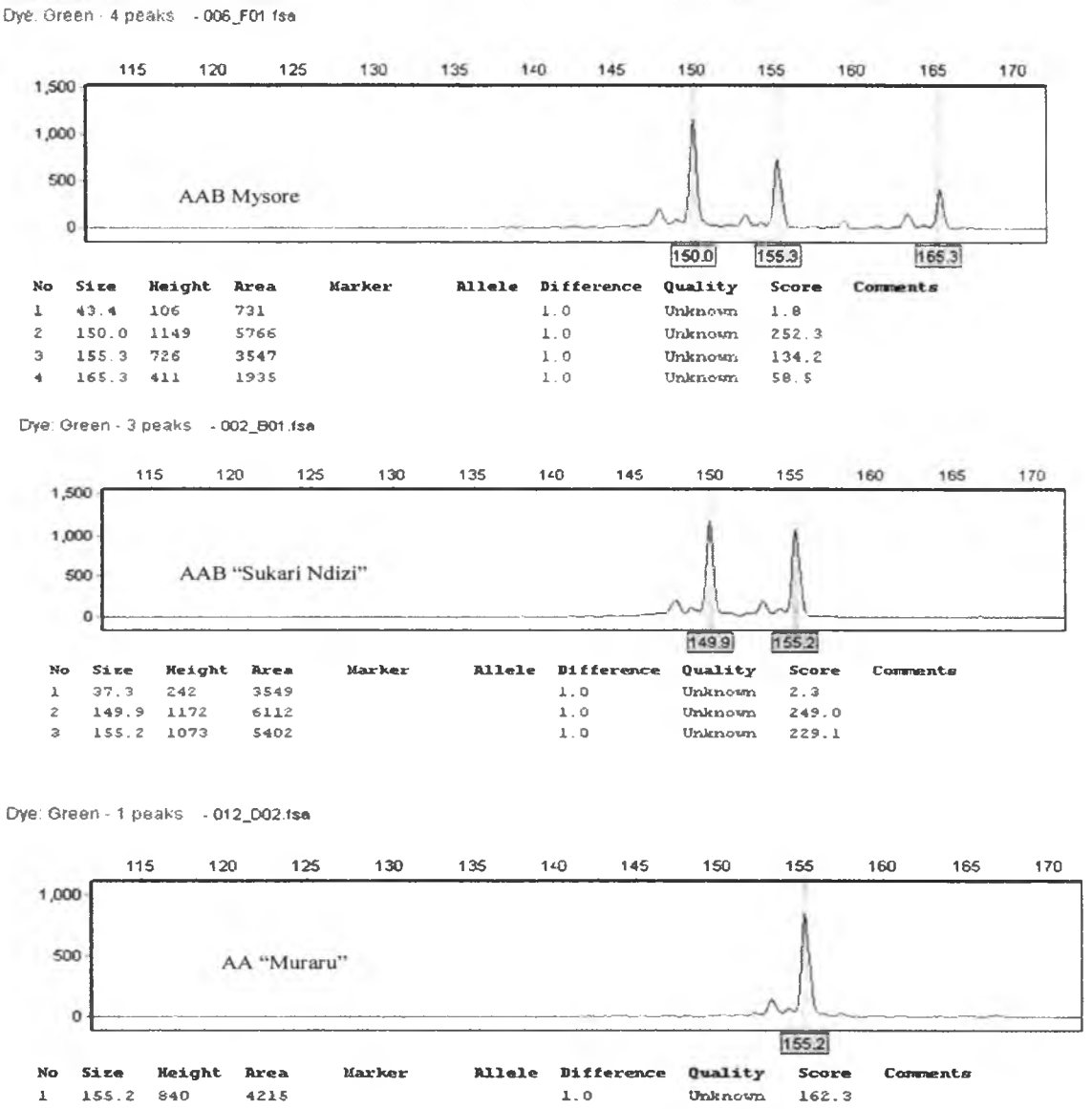
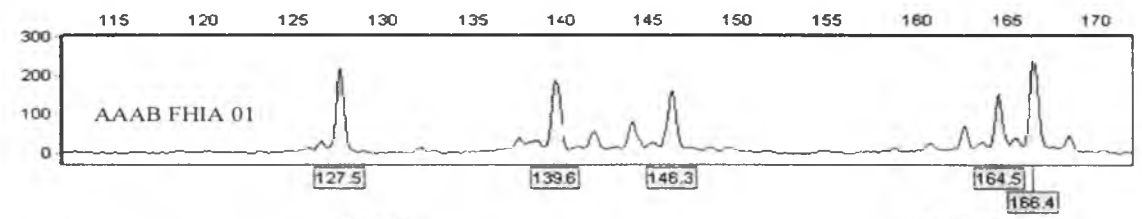


Figure 4. 4: Allele reports for SSR primer ma3-103 for three representative banana accessions. Each x-axis shows fragment size in basepair and y-axis show height of fragment peak. AAB Mysore has three alleles, AAB "Sukari Ndizi" has two alleles and AA "Muraru" has one allele at this locus.

Sample 52:

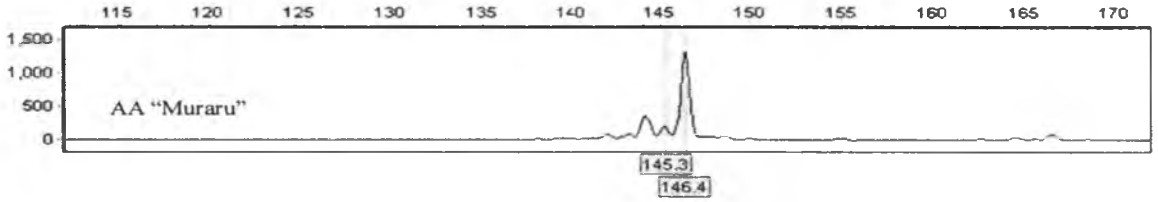
Dye: Blue - 6 peaks - 052_D07.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	38.2	152	998			1.0	Unknown	4.4	
2	127.5	218	1112			1.0	Unknown	17.9	
3	139.6	187	1046			1.0	Unknown	11.8	
4	146.3	158	885			1.0	Unknown	8.6	
5	164.5	153	782			1.0	Unknown	9.6	
6	166.4	238	1191			1.0	Unknown	21.5	

Sample 55:

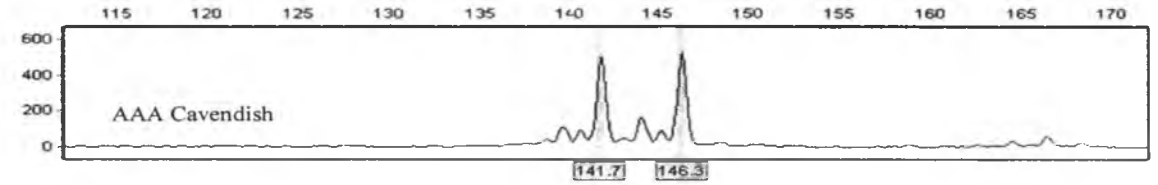
Dye: Blue - 3 peaks - 055_G07.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	38.3	144	961			1.0	Unknown	3.2	
2	145.3	203	1239			1.0	Unknown	9.4	
3	146.4	1324	7223			1.0	Unknown	276.1	

Sample 62:

Dye: Blue - 3 peaks - 062_F08.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	38.2	150	924			1.0	Unknown	3.8	
2	141.7	501	2928			1.0	Unknown	57.2	
3	146.3	523	2878			1.0	Unknown	67.6	

Figure 4. 5: Allele reports for SSR primer ma3-139 for three representative banana accessions. Each x-axis shows fragment size in basepair and y-axis show height of fragment peak. AAAB FHIA 01 has four alleles, AA "Muraru" has one allele and AAA Cavendish has two alleles at this locus.

Table 4.6 gives the results of the number of the alleles obtained from each of the 8 SSR markers. Marker Ma3-90 gave the highest number (15) of SSR alleles while marker Ma3-60 had the lowest number (3). This set of discrete characters was used for further analysis.

Table 4. 6: Allele numbers for each nuclear SSR marker used and the size ranges

SSR marker	Number of alleles	Range of allele sizes
Ma1-16	12	157 - 189
Ma1-27	6	122 - 140
Ma3-48	8	140 - 169
Ma3-60	3	103 -115
Ma3-90	15	132 - 163
Ma3-103	11	150 - 171
Ma3-104	14	137 - 166
Ma3-139	11	126 - 167
Total	80	103 - 189

4.3.2. Genetic similarity

Cluster analysis of the binary data from the 80 SSR alleles (Table 4.6) across all 133 banana accessions produced the phenograms shown in Figures 4.6 and 4.7. The cluster analyses (Figures 4.6 to 4.13) and PCAs (Figures 4.14 to 4. 20) separated the 133 accessions into major subgroups corresponding with the genome designation of the accessions (i.e., AAA, AAB, ABB, AB, AA, BB). Phenograms derived from similarity analyses, based on Simple Matching (Figure 4.6) or Jaccard's (Figures 4.7 to 4.13) coefficients, showed robust clustering of the various banana subgroups. The

Simple Matching coefficient, based on shared presence and absence of alleles gave higher similarity values (1 to 0.78) compared to Jaccard's coefficient (1 to 0.22), which is based on only the shared presence of alleles. Regardless of which similarity coefficient was employed, the grouping of related accessions changed little although minor differences in relationships between groups did occur. The phenogram based on Jaccard's coefficient indicates that the four major groups of AAB Apple bananas are more closely related to one another than to the AAA Cavendish and Gros Michel dessert banana group, whereas the phenogram based on the Simple Matching coefficient places the "Sukari Ndizi" and Prata bananas closer to the AAA dessert group compared to the other AAB Mysore and Silk groupings.

There was satisfactory agreement ($r \geq 0.9$) of the phenograms with their similarity matrix, an indication of reliability of the phenograms. Figure 4.8 is a phenogram for AAB, AB and AAAB groups composed of 70 banana accessions while Figure 4.9 presents only AAB Apple banana accessions (53) with their 2 tetraploid hybrids (FHIA 01 and GT) and AB accessions. The cluster analysis in Figure 4.10 is a phenogram based on 54 accessions of only *acuminata*-based accessions (AAA and AA groups). Figure 4.11 is a CA composed entirely of the AA group with 31 accessions. Figures 4.12 and 4.13 are phenograms of ougroups AAA only and BB, AB, and ABB only respectively.

The average similarity (Table 4.7) among the AAB "Apple" based on Jaccard's coefficient ranged from 0.333 (Silk and Mysore) to 0.647 ("Sukari Ndizi" and Prata). Within the AAB "Apple" subgroup, Silk presented the lowest similarity values compared to "Sukari Ndizi", Prata and Mysore. Generally the highest

similarity values were observed within sub-groups. High similarity (0.733) was found between "Sukari Ndizi" and GT – a known natural hybrid of "Sukari Ndizi" and Gros Michel or "Muraru". The similarity of FHIA01 and the Prata accessions was also high (0.750), corroborating that FHIA01 is a hybrid of Prata ana. The dessert cultivars, namely, the Cavendish group, Gros Michel, Prata, and "Sukari Ndizi" all had high similarities (>0.500) with each other. The AAB plantains and AAA East African highland bananas, however, had relatively low similarity (< 0.400) between themselves and to other sub-groups.

The clusters based on the Simple Matching coefficient (Table 4.8) were in agreement with those of Jaccard's coefficient (Table 4.7). The Simple Matching coefficient, nevertheless, gave higher relative similarity values implying that accessions in this study were more closely related. The East African AA "Muraru" had a high relative similarity (>0.9) to the AAA Gros Michel and Cavendish dessert bananas, and (>0.8) to the AAB "Sukari Ndizi" dessert bananas. The two East African dessert bananas ("Sukari Ndizi" and "Muraru") had the highest relative similarity to the commercial export AAA bananas. Of great interest was accession AA Kamunyilya, which was in the same cluster as AAA Gros Michel and had a similarity of 0.96. The "Muraru," however, have a low relative similarity (<0.3) on Jaccard's coefficient and <0.85 on Simple Matching coefficient] to plantains and East African cooking bananas.

4.3.3. Cluster analysis

In Figure 4.6, based on Jaccard's similarity index, the 8 markers divided the accessions into 5 distinct clusters: 1) the AAB "Apple" dessert, 2) AAB plantain, 3) the purely *acuminata* dessert bananas consisting of AAA Cavendish, AAA Gros Michel, and AA "Muraru," 4) AAA East African Highland cooking bananas (AAAEA), and 5) the Sucrier and related AA dessert bananas. The clusters were further subdivided into sub-clusters representing different banana subgroups. The AAB "Apple" bananas accessions formed one big cluster within which there are four taxa; the AAB Mysore, the AAB "Sukari Ndizi," the AAB Prata and the AAB Silk (Figures 4.6 and 4.7). The tetraploid hybrids AAAB FHIA01 and AAAB GT clustered with AAB Prata and AAB "Sukari Ndizi," respectively.

In Figure 4.7, based on Simple Matching index, the distinction between AAB "Apple" and AAA and AA "Muraru" dessert bananas, but the other three groups retain their identities.

Figure 4.8 is a phenogram from UPGMA of Jaccard's similarity coefficient respectively, for all the AAB groups and AB groups, and two tetraploid AAAB hybrids. Figure 4.9 is a phenogram for only the AAB "Apple" accessions. The analysis separates the four AAB "Apple" banana taxa from that of the AAB plantains and Polynesian bananas.

The results showed that the Silk cluster included accessions ITC348 Silk, ITC1222 Sports of Silk, ITC769 Figue Pomme Geante, and AB accessions like ITC245Safet Velchi and Kisubi. This cluster had two distinct sub-groups; one contained the AAB Silk and the AB and ABB groupings combined (Figure 4.6). The

following accessions from East Africa were found in this cluster of AAB Silk: Pukusa from Zanzibar, and Ungoye, Manjano, and Mboki Msukari from the Kisii collection. The AB Kisubi and AB Safet Velchi, together with the ABB Kayinja, formed the second sub-clusters in this cluster. Gisubi, a triploid from Rwanda, was distinct but closest to this cluster.

The 2nd major cluster consisted of AA "Muraru" accessions, the Gros Michel and Cavendish dessert bananas (figures 4.6 and 4.7). Within this cluster there were three sub-clusters representing these three taxa of bananas. The triploid AAA Gros Michel and AAA Cavendish clustering together in the big cluster and in addition the SSR markers were able to separate these two AAA dessert bananas taxa. A few AA reference accessions (Sucrier) which were probably cultivated types formed a third and separate cluster by themselves, near the wild AA bananas (Figures 4.6 and 4.7). Accession Datil formed a cluster with Kipaka, Kirun, Ngu, Sucrier, and Hapai, an indication that Datil is a diploid AA and not AB as recorded. A separate study with flow cytometry corroborates this conclusion.

The fourth and fifth major clusters were formed by the two distinct cooking bananas, i.e., plantain and East African highland cooking bananas. The East African highland bananas clustered together as one group, while the three African plantains and nine Polynesian plantains clustered together as one unit with the Bioversity International AAB reference accessions, ITC0987 Auko and ITC0990 Vunapope as well. The ABB Bluggoe, Ngoja and Dipping formed a cluster close to the plantains. AA diploids ITC0428 Higa Banksii, ITC1253 Mjenga Michel diploid, ITC0435 Pisang Mas Ayer ITC0480 Pisang Buntal and ITC0672 Pa Rayong and AB diploid

ITC1034 Kunnan and were close to the plantain cluster. The other phenograms (Figures 4.8 to 4.11) of fewer accessions based on genomic groups had clusters consistent with the overall clustering relationships. Allele 150 of primer pair Ma3-103 could be a private allele for Apple banana accessions. This marker can thus be used for diagnosis purposes, and any plant that showed allele 150bp must contain B genome and is likely an "Apple."

Figures 4.12 and 4.13 are phenograms showing relationships found in other groups used as outgroup namely AAA group and ABB, BB, and AB groups.

Table 4. 1: Average similarity among the banana sub-groups derived from Jaccard's similarity correlation coefficient

Group	**Mysore**	**"Sukari Ndizi"**	**Prata**	**Silk**	**"Muraru"**	**Cavendish**	**Gros Michel**	**AAB plantains**	**AAA cooking**
Mysore	X								
"Sukari Ndizi"	0.474	X							
Prata	0.429	0.647	X						
Silk	0.333	0.625	0.556	X					
"Muraru"	0.300	0.563	0.588	0.333	X				
Cavendish	0.391	0.529	0.526	0.318	0.556	X			
Gros Michel	0.318	0.500	0.450	0.286	0.529	0.579	X		
AAB plantains	0.200	0.333	0.409	0.318	0.273	0.280	0.200	X	
AAA cooking	0.240	0.318	0.292	0.217	0.286	0.280	0.200	0.280	X

Table 4. 2: Average similarity among the banana sub-groups derived from Simple Matching similarity correlation coefficient

Group	**Mysore**	**"Sukari Ndizi"**	**Prata**	**Silk**	**"Muraru"**	**Cavendish**	**Gros Michel**	**AAB plantains**	**AAA cooking**
Mysore	X								
"Sukari Ndizi"	0.875	X							
Prata	0.850	0.925	X						
Silk	0.825	0.925	0.900	X					
"Muraru"	0.830	0.913	0.913	0.863	X				
Cavendish	0.825	0.900	0.875	0.825	0.913	X			
Gros Michel	0.838	0.863	0.863	0.813	0.900	0.913	X		
AAB plantains	0.750	0.825	0.825	0.813	0.813	0.788	0.750	X	
AAA cooking	0.763	0.813	0.788	0.763	0.800	0.763	0.750	0.775	X

4.3.4. Principal component analysis (PCA)

The PCA analysis showed that 86.1% of the total variability in the 8 SSR markers is captured in the first two principal axes (The first three principal axes include 88.4% of the variability). The PCA (Figure 4.14) showed separation of accessions composed only of *acuminata* from those with *balbisiana* genome, while the PCA (Figure 4.15) showed distinct groupings for the three major groups, the cooking type (AAB Plantains and AAA cooking bananas) and Dessert types (AAA desserts, Cavendish, Gros Michel and "Muraru", AAB "Apple" dessert bananas, Prata, "Sukari Ndizi," Mysore and Silk). Figures 4.16 to 4.19 are based on only 103 accessions that were considered cultivated bananas. Various patterns were obtained using PCA based on various combinations of the principal axes. Except for the non-cultivated diploid banana accessions, the PCA separated the subjectively classified cooking bananas (AAB Plantains and AAA cooking) from the dessert bananas (AAA Gros Michel and Cavendish, and AAB "Apple" dessert bananas) as shown in Figures 4.15, 4.17, and 4.18. Thus, regardless of genomic groupings, these separations agreed with the subjective classification of usage. Figure 4.14 showed a separation of the accessions on the basis of the presence of B in the genome of accession while principal axes 1 still separating on the subjective classification of usage, cooking types on the left and dessert types on the right. The only exceptions for principal axes 1 were the non- cultivated diploids that were nonetheless still separated by principal axis 3 on the basis of containing B genome.

Figure 4.14 show principal axes 3 separating banana accessions with B from those with A only. Figures 4.15 and 4.17 show the separations of the accessions in

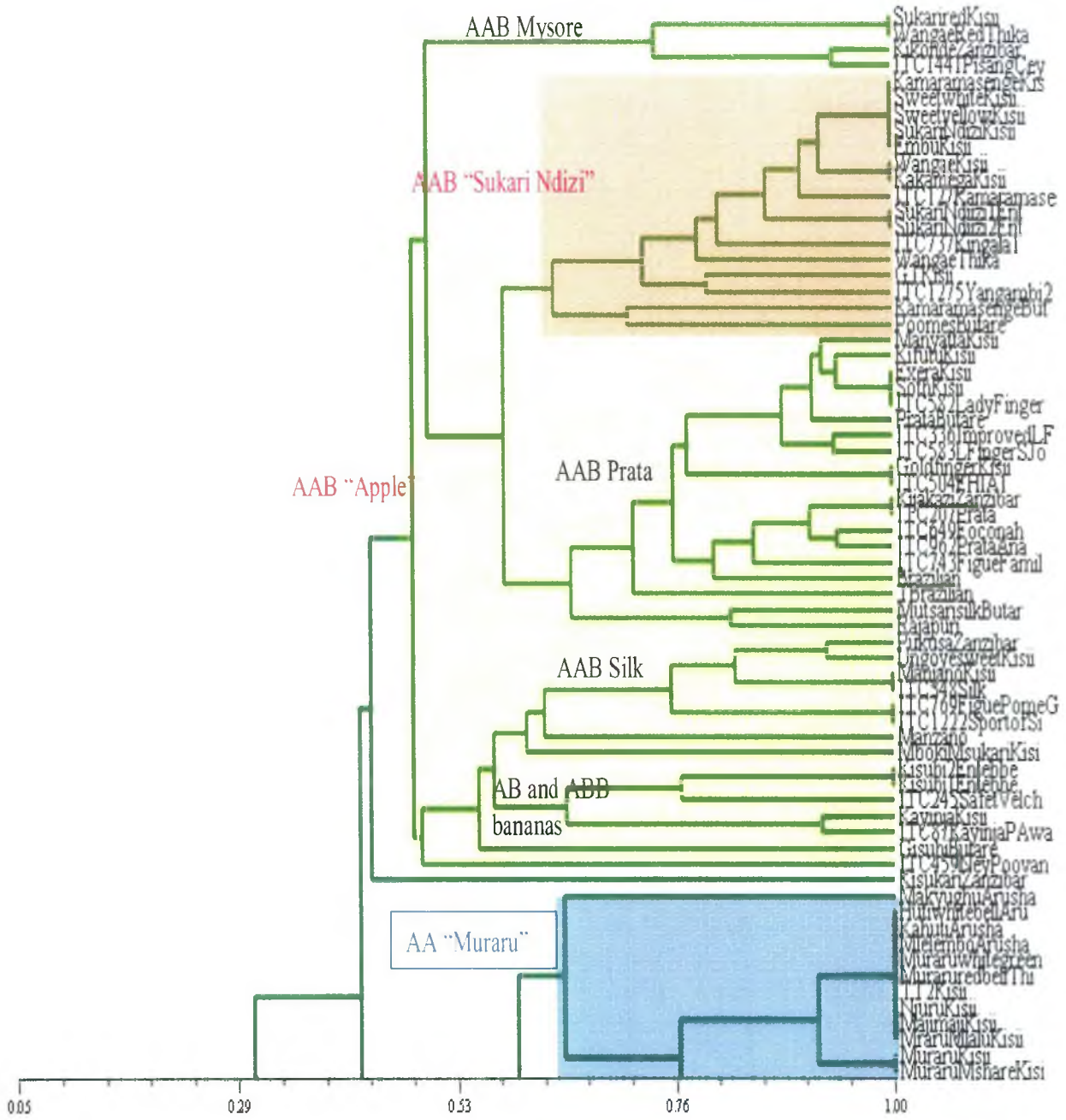


Figure 4. 6: Phenogram from UPGMA clustering using Jaccard's similarity coefficient among 133 banana accessions including AAB "Apple" and AA "Muraru" and outgroups based on microsatellite markers.
Cophenetic value = 0.892.

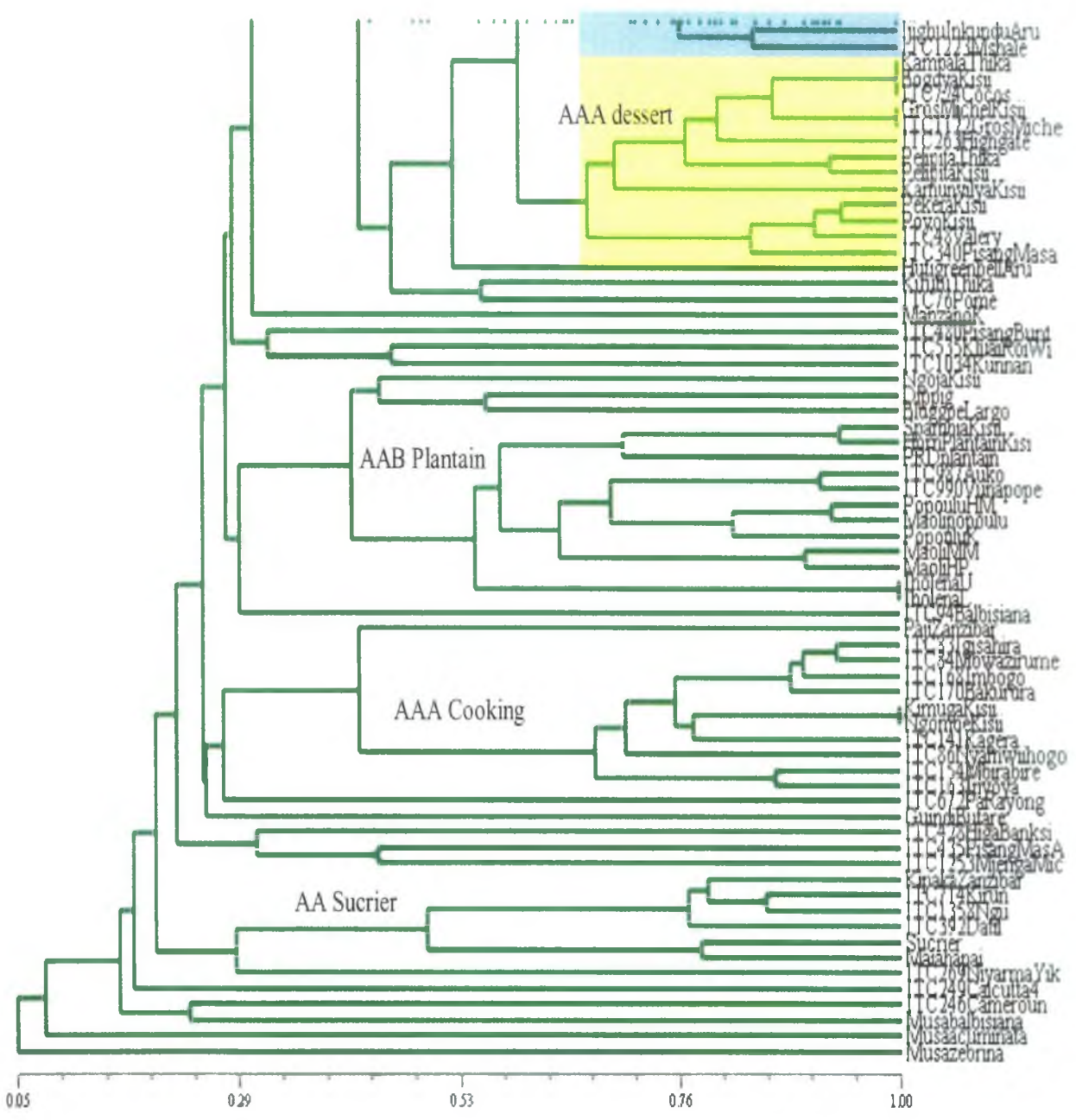


Figure 4.6: Phenogram from UPGMA clustering using Jaccard's similarity coefficient among 133 banana accessions including AAB "Apple" and AA "Muraru" and outgroups based on microsatellite markers.
Cophenetic value = 0.892.

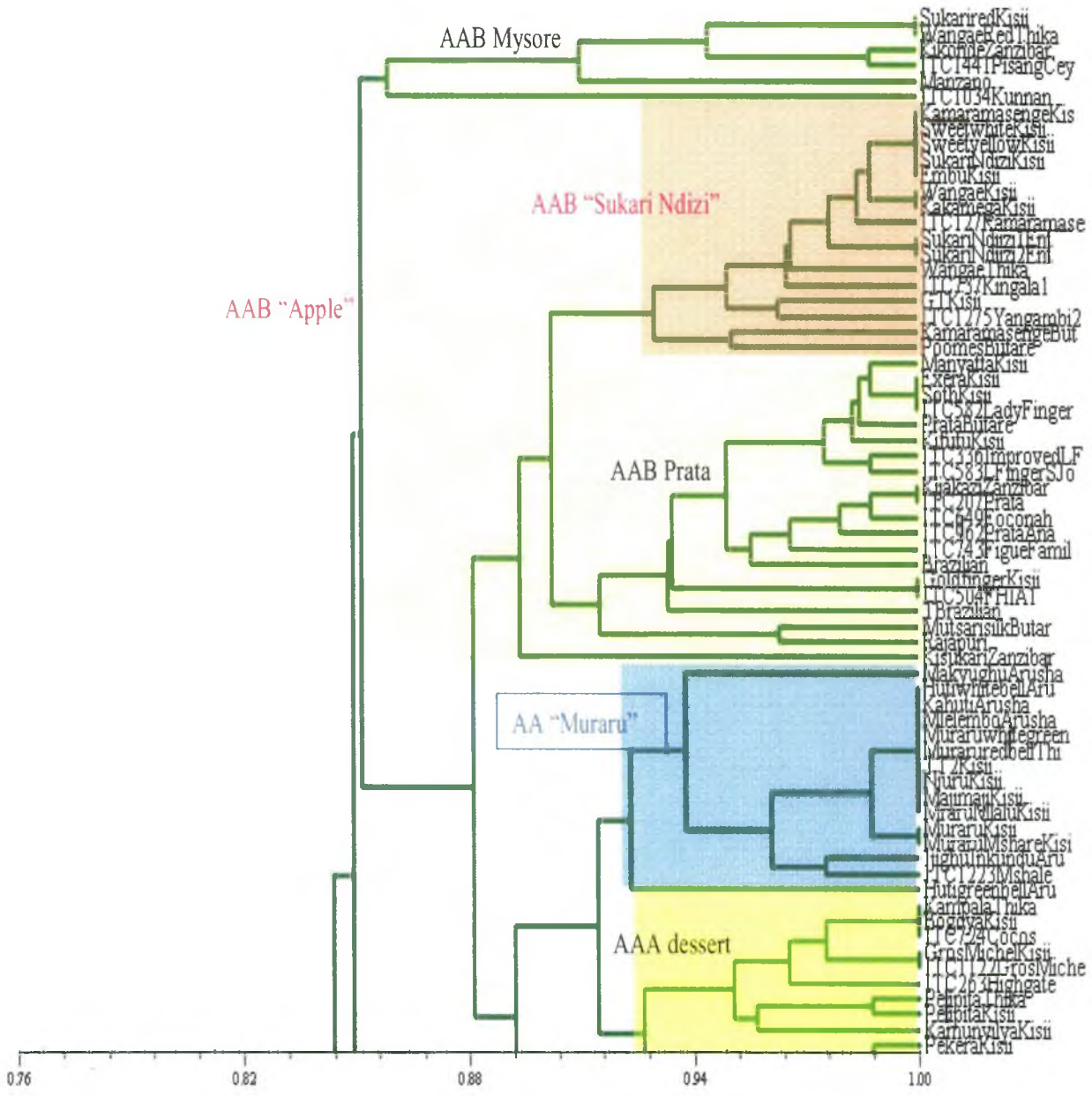


Figure 4. 7: Phenogram from UPGMA clustering using Simple Matching similarity coefficient among 133 banana accessions including AAB "Apple" and AA "Muraru" and outgroups based on microsatellite markers.
Cophenetic value = 0.854.

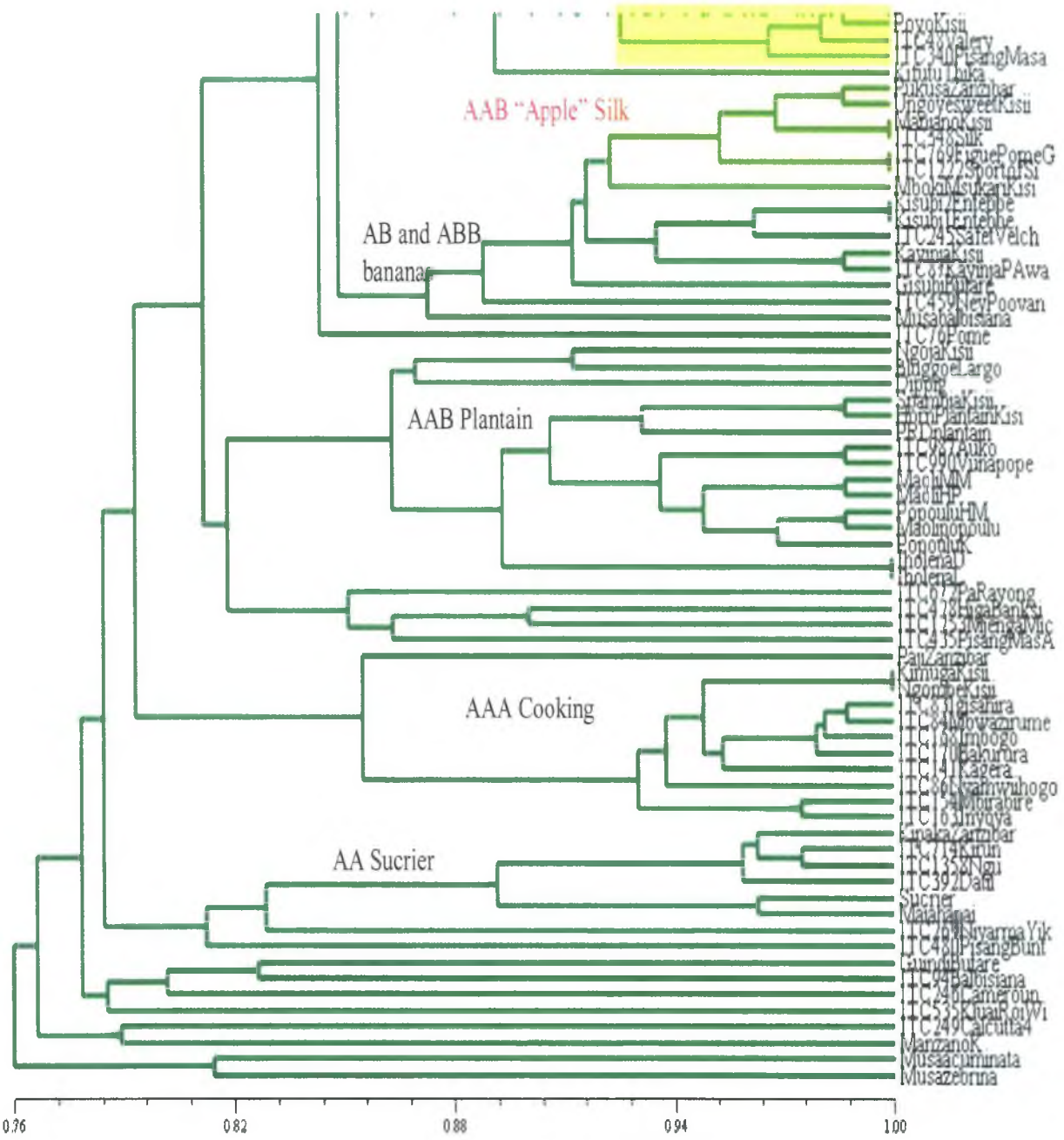


Figure 4.7: Phenogram from UPGMA clustering using Simple Matching similarity coefficient among 133 banana accessions including AAB "Apple" and AA "Muraru" and outgroups based on microsatellite markers.
Cophenetic value = 0.854.

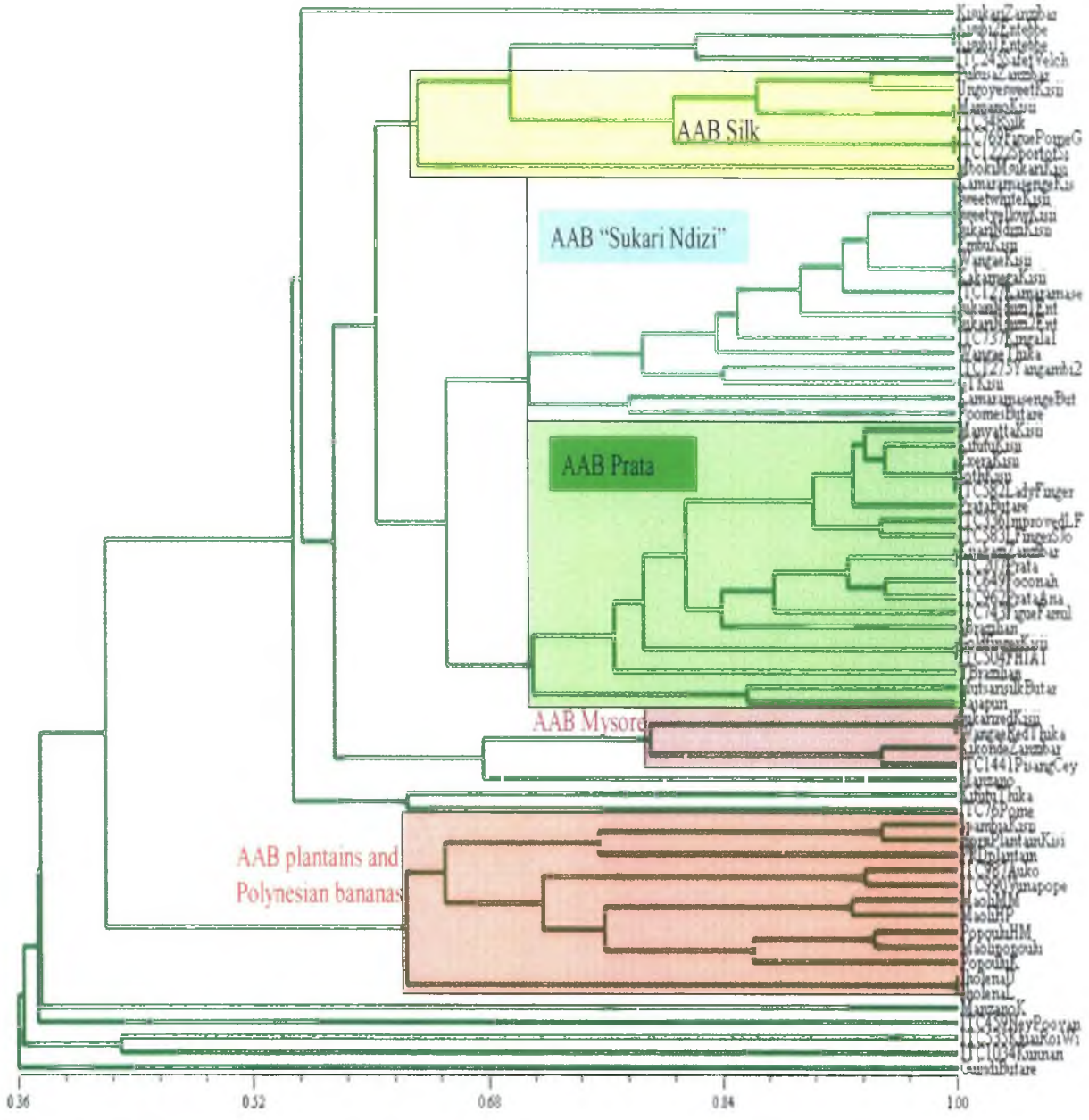


Figure 4. 8: Phenogram from UPGMA clustering using Jaccard's similarity coefficient for all 70 accessions in AB and AAB Groups and their AAAB derivatives, based on microsatellite markers.
Cophenetic value = 0.916.

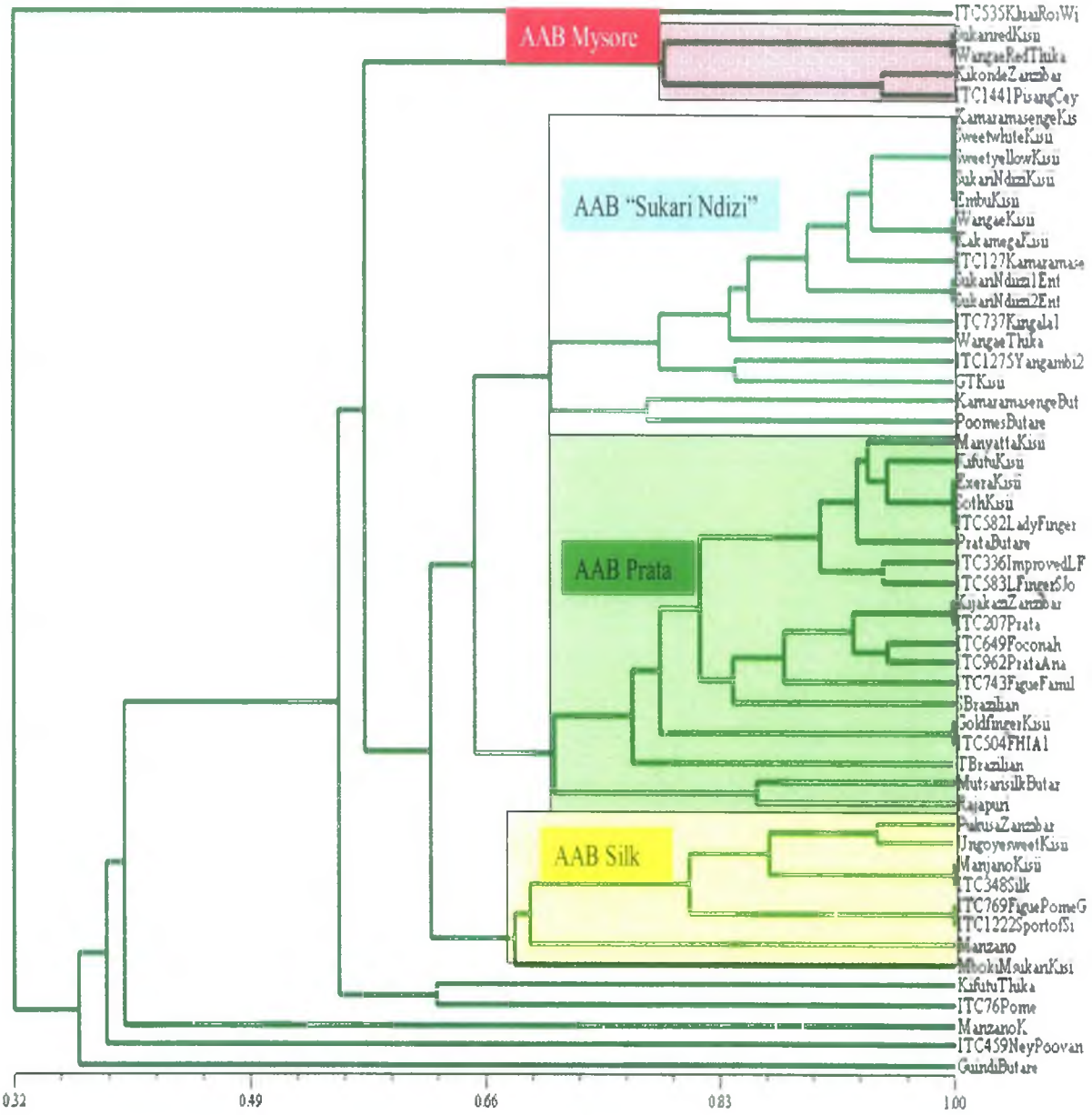


Figure 4. 9: Phenogram from UPGMA clustering using Jaccard's similarity coefficient for all 51 AAB "Apple" accessions and their AAAB derivatives based on microsatellite markers. Cophenetic value 0.900.

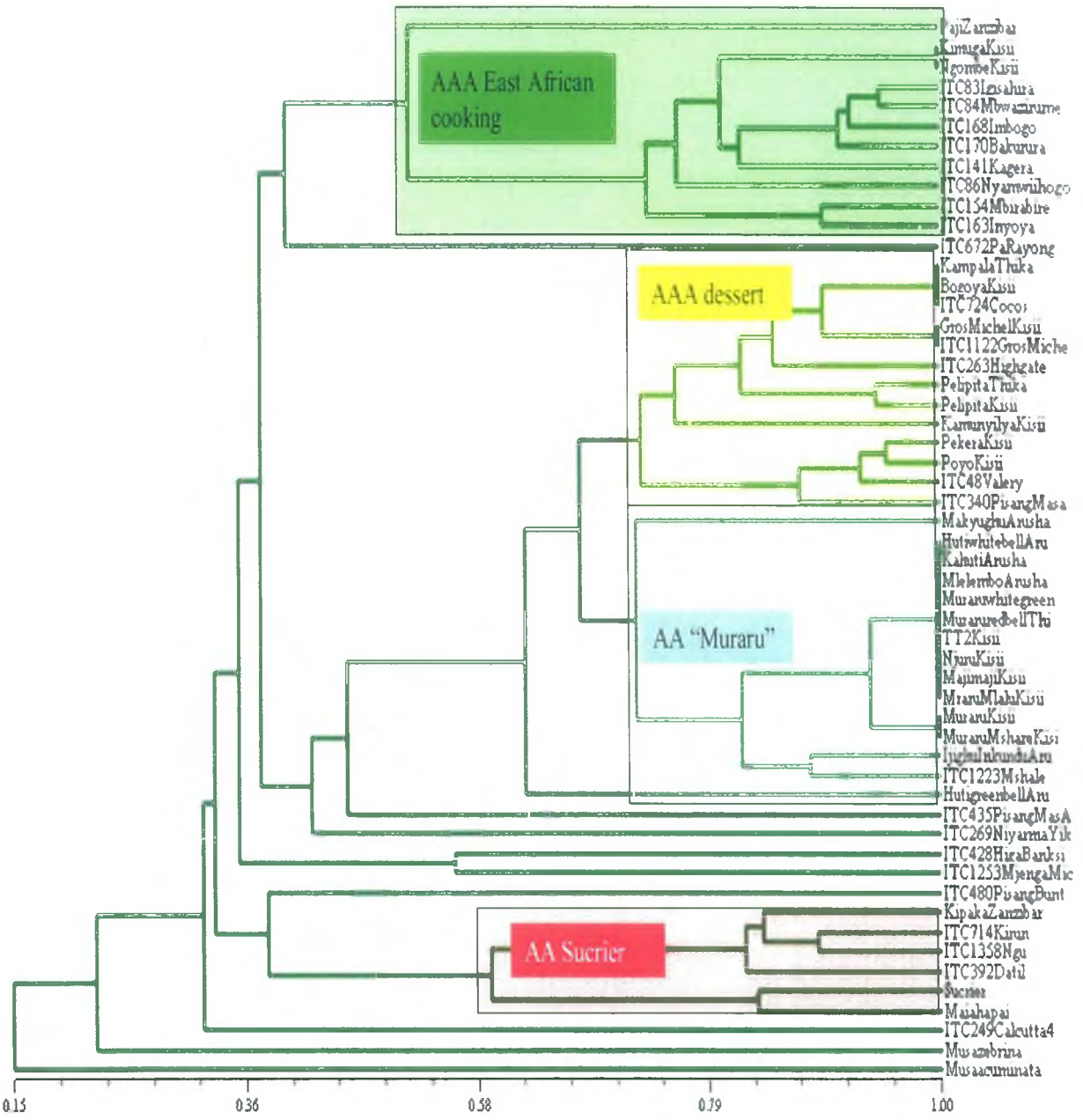


Figure 4. 10: Phenogram from UPGMA clustering using Jaccard's similarity coefficient for all 54 accessions of AAA and AA groups based on microsatellite markers. Cophenetic value = 0.900.

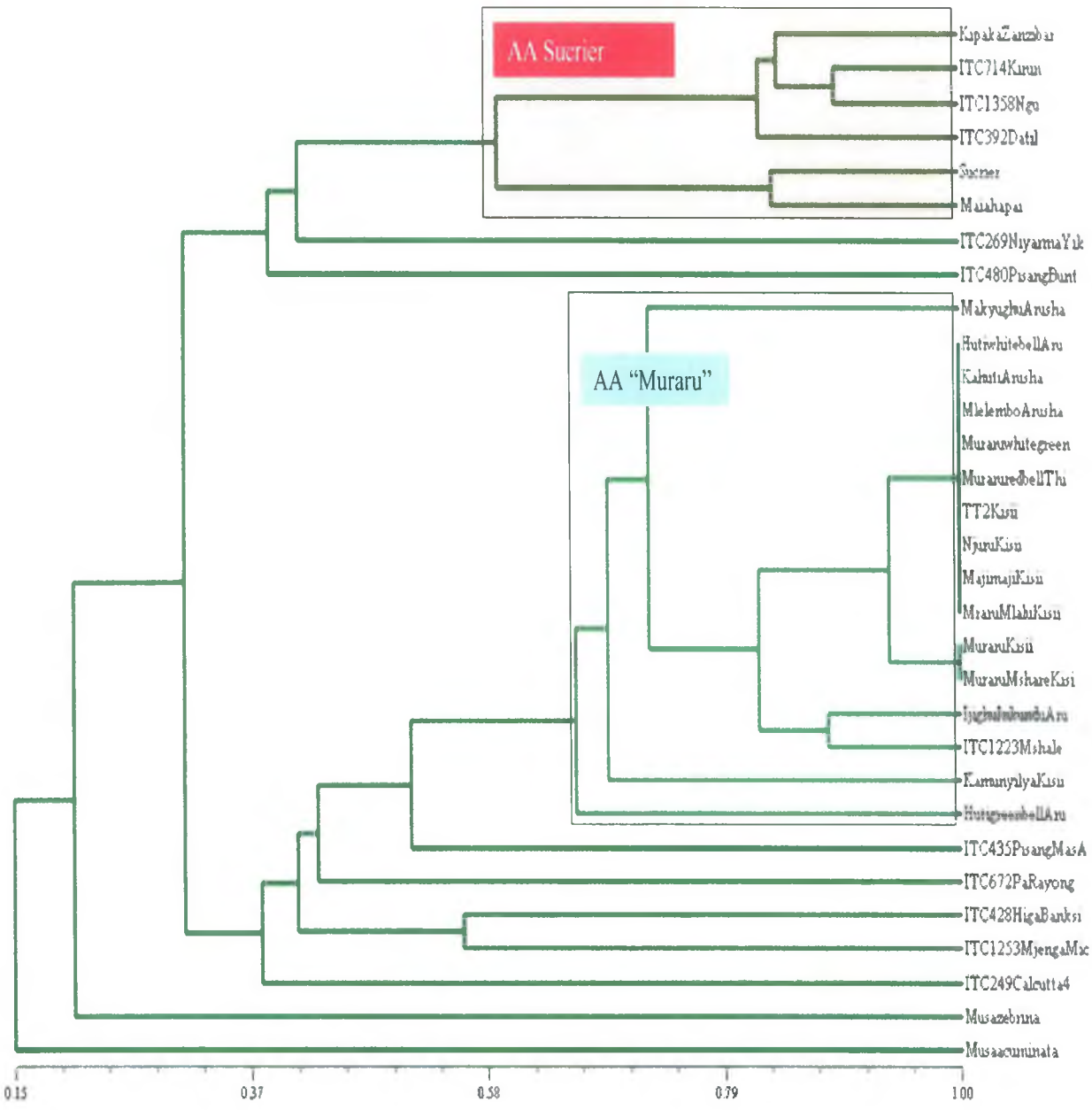


Figure 4. 11: Phenogram from UPGMA clustering using Jaccard's similarity coefficient for all 31 accessions in AA group based on microsatellite markers. Cophenetic value = 0.900.

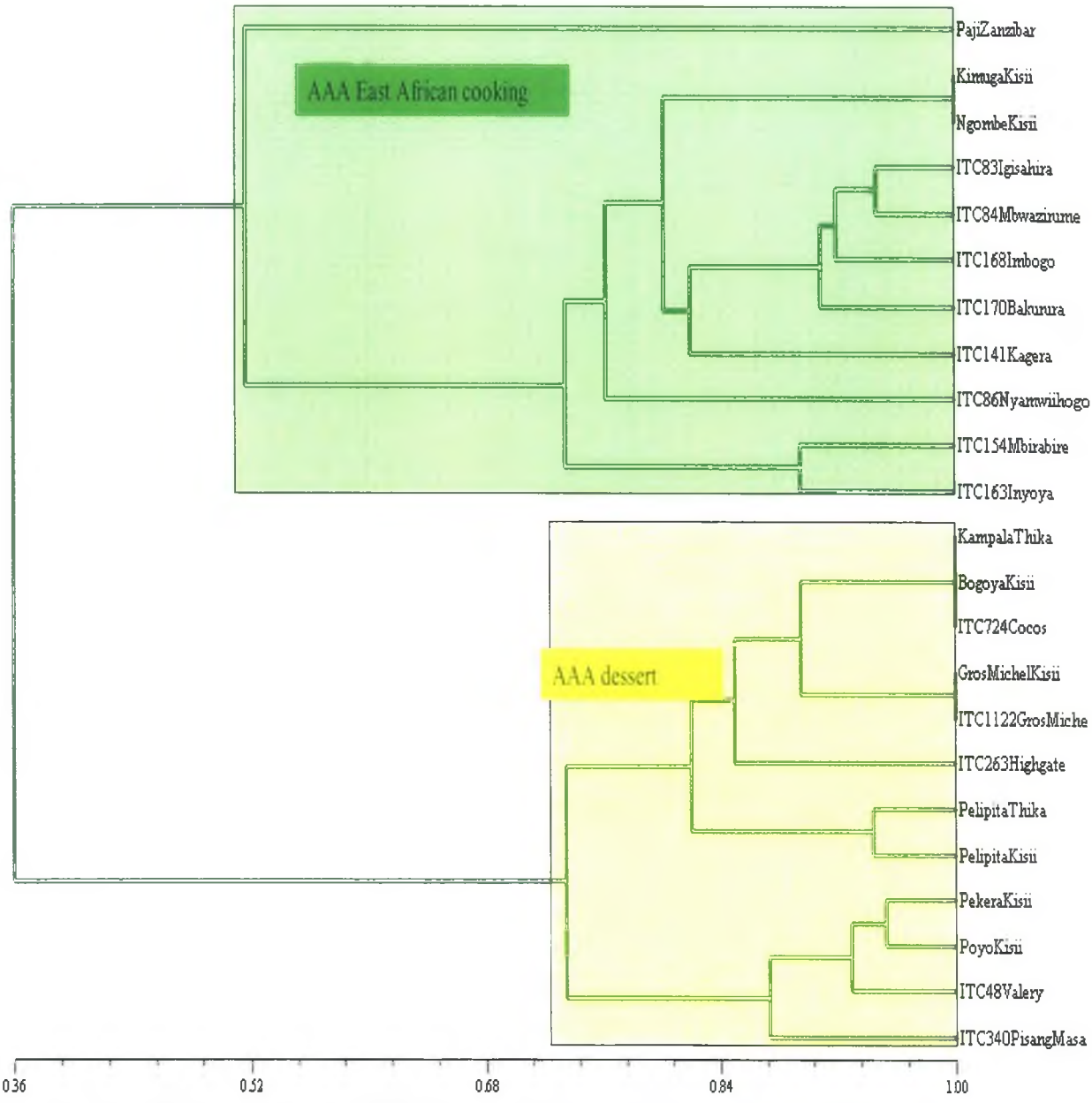


Figure 4. 12: Phenogram from UPGMA clustering using Jaccard's similarity coefficient for all 23 accessions in AAA group based on microsatellite markers. Cophenetic value = 0.900.

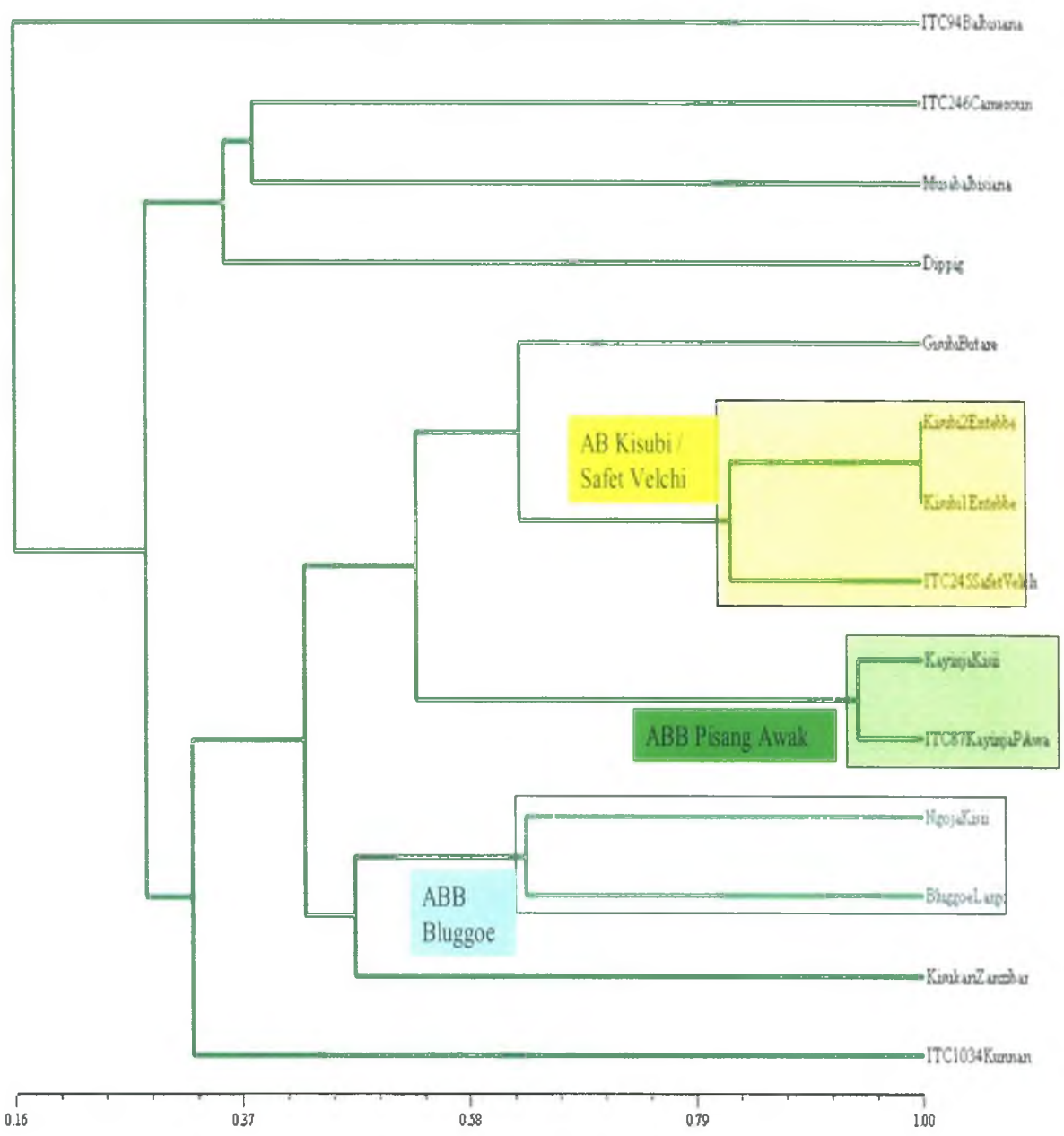


Figure 4. 13: Phenogram from UPGMA clustering using Jaccard's similarity coefficient for all 14 accessions in BB, AB, AND ABB groupS based on microsatellite markers.
Cophenetic value = 0.913.

agreement with the subjective classification based on use (the dessert bananas are separated from cooking bananas regardless of genomic composition). Further scrutiny of the various PCAs, (Figures 4.14, 4.15, 4.17, 4.18, and 4.19) showed that the accessions have been separated into genomic groups. Figure 4.18 appears to separate all of the banana accessions into subgroups; though there is an overlap because of shared genes, the isolation into various units was very clear overal.

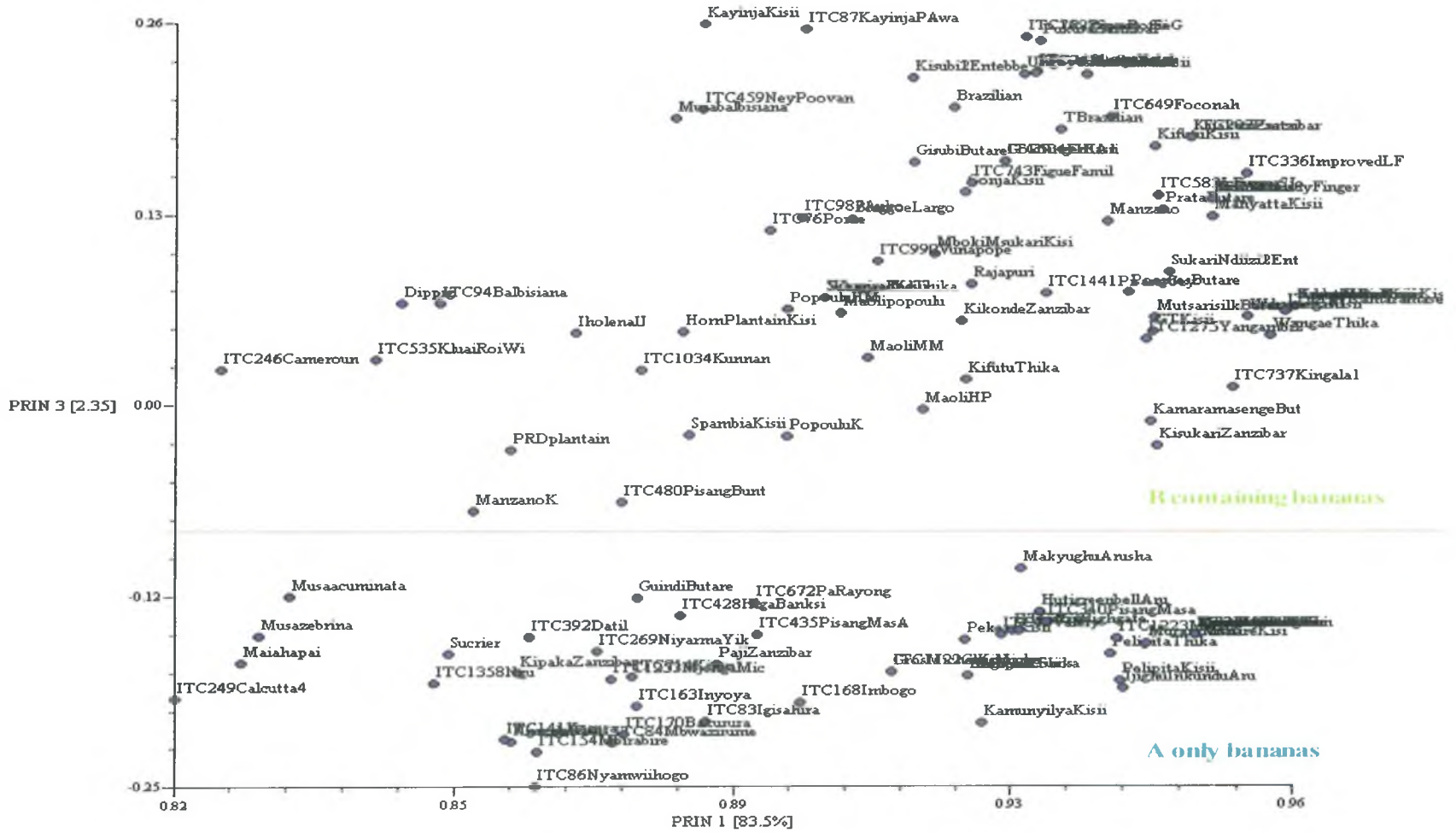


Figure 4. 1: PCA showing relative positions on the 1st and 3rd principal axes of 133 accessions, based on SSR markers: AAB "Apple" and AA "Muraru" and all outgroups. Notice the separation (red line) of B containing accessions from those with A only genomes by principal axes 3.

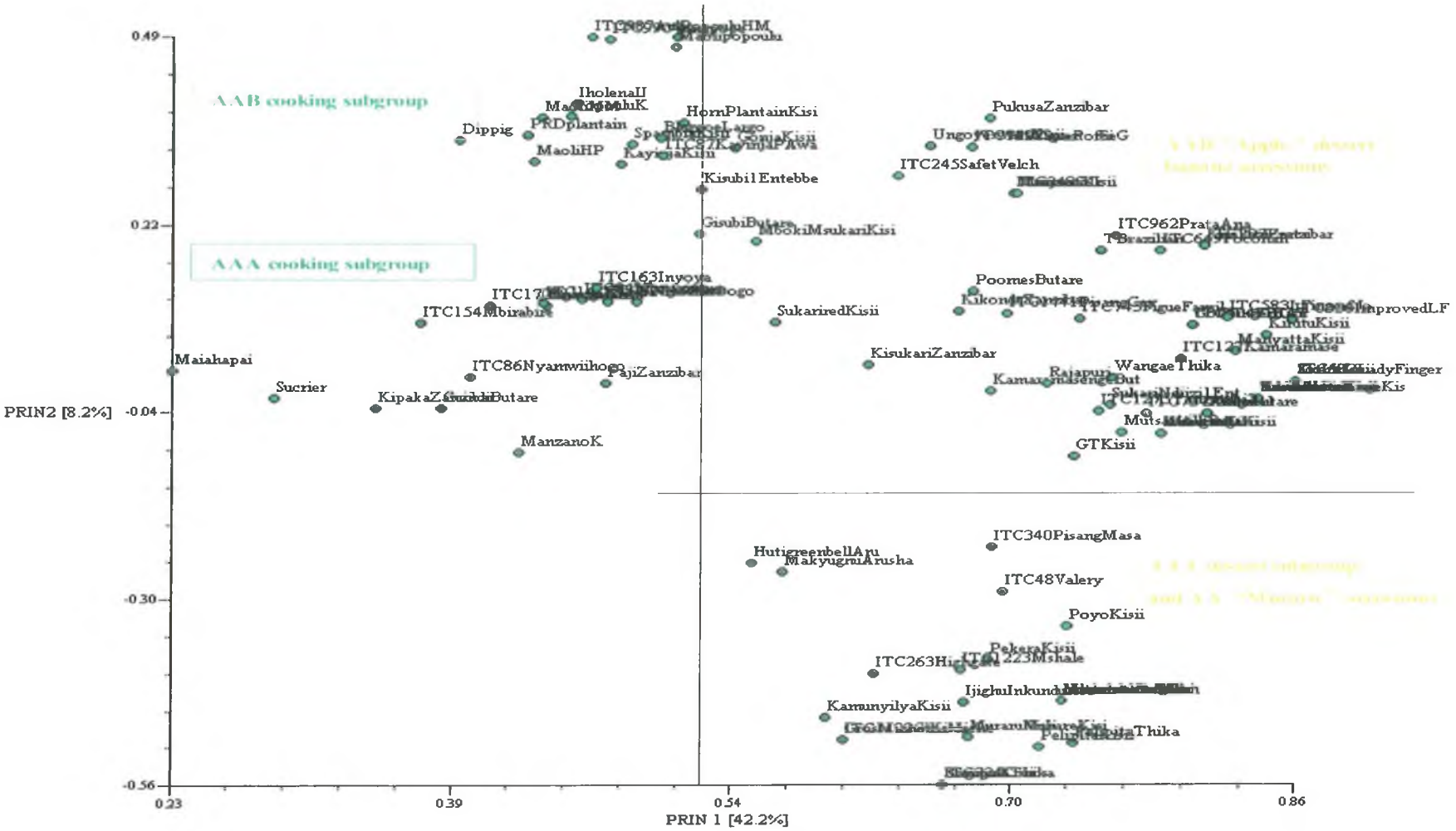


Figure 4. 2: PCA showing relative positions on the 1st and 2nd principal axes of 103 cultivated banana accessions, AAB "Apple" and AA "Muraru" and selected cultivated outgroups.

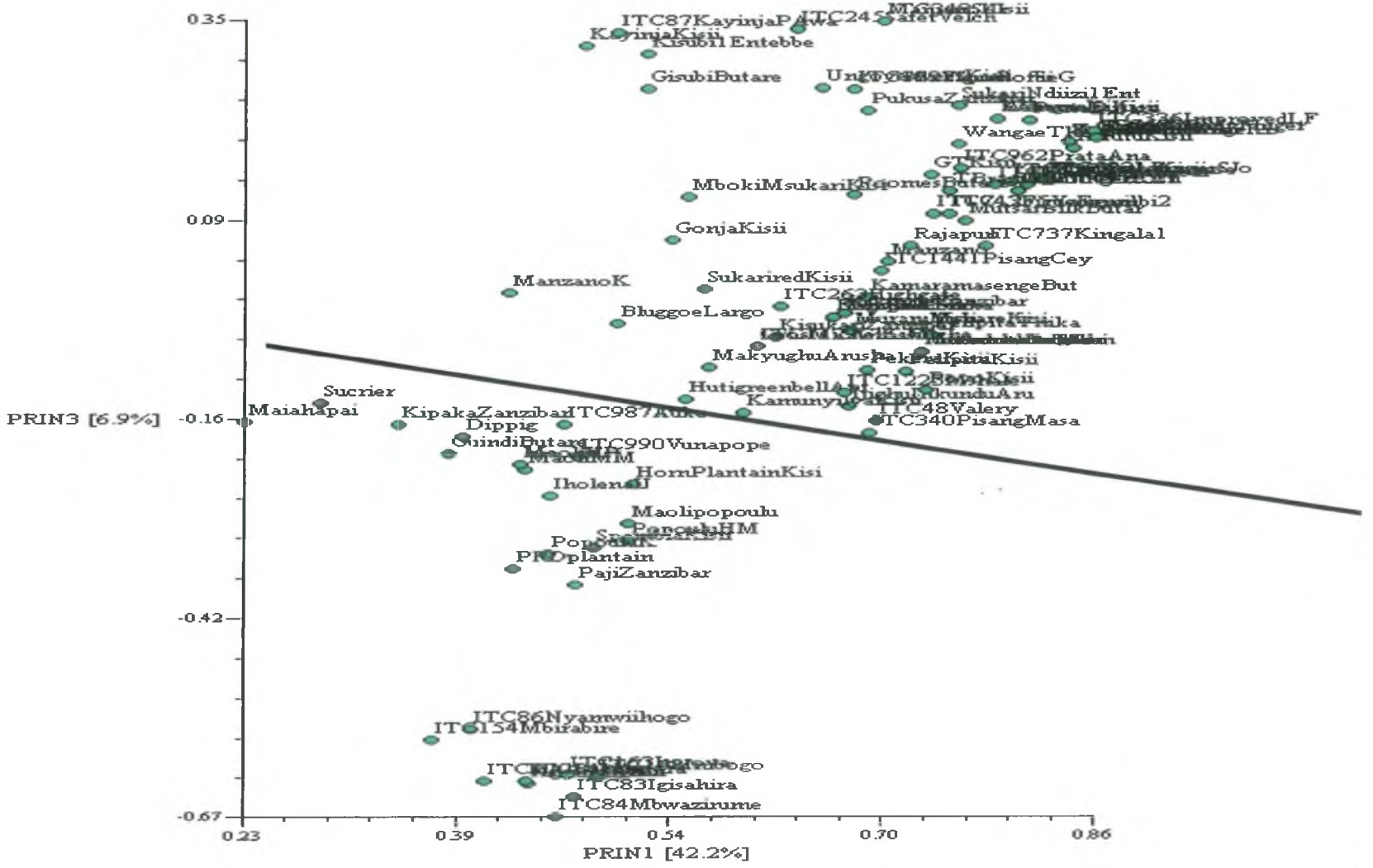


Figure 4. 3: PCA showing relative positions on the 1st and 3rd principal axes of 103 cultivated banana accessions, AAB "Apple" and AA "Muraru" and selected cultivated outgroups.

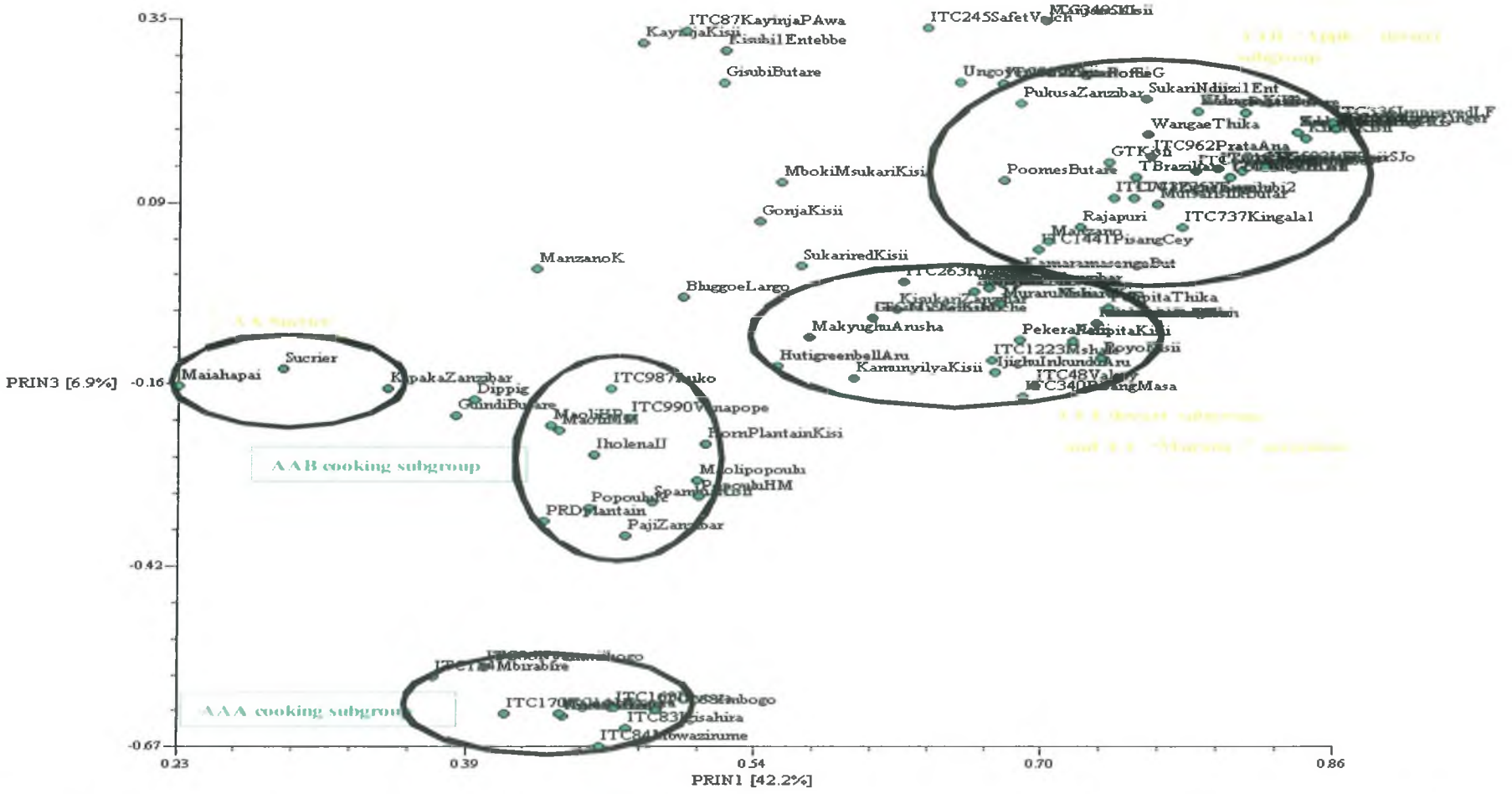


Figure 4. 4: PCA showing relative positions on the 1st and 3rd principal axes of 103 cultivated banana accessions, AAB "Apple" AA and "Muraru" and selected cultivated outgroups.

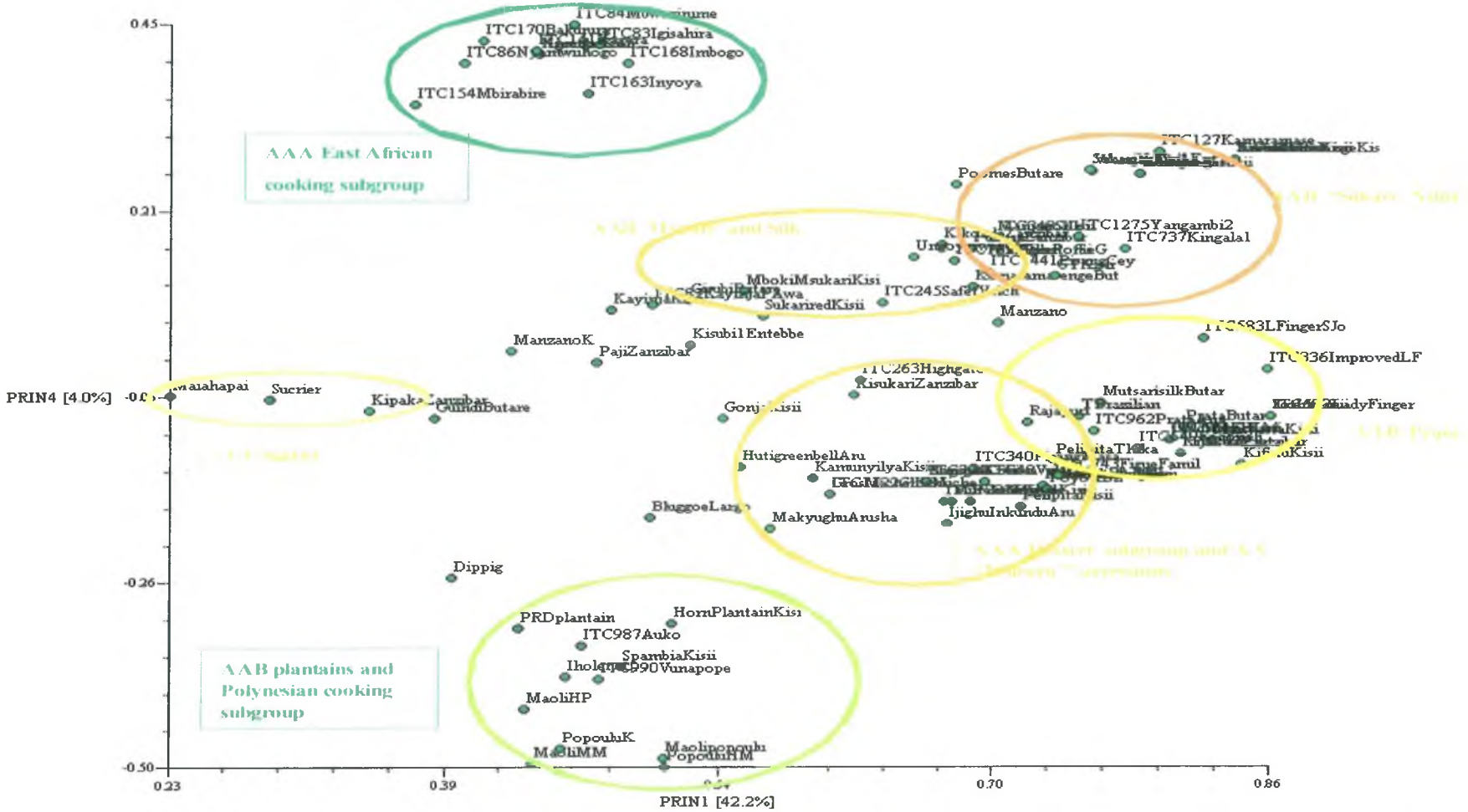


Figure 4. 5: PCA showing relative positions on the 1st and 4th principal axes of 103 cultivated banana accessions, AAB "Apple" and AA "Muraru" and selected cultivated outgroups.

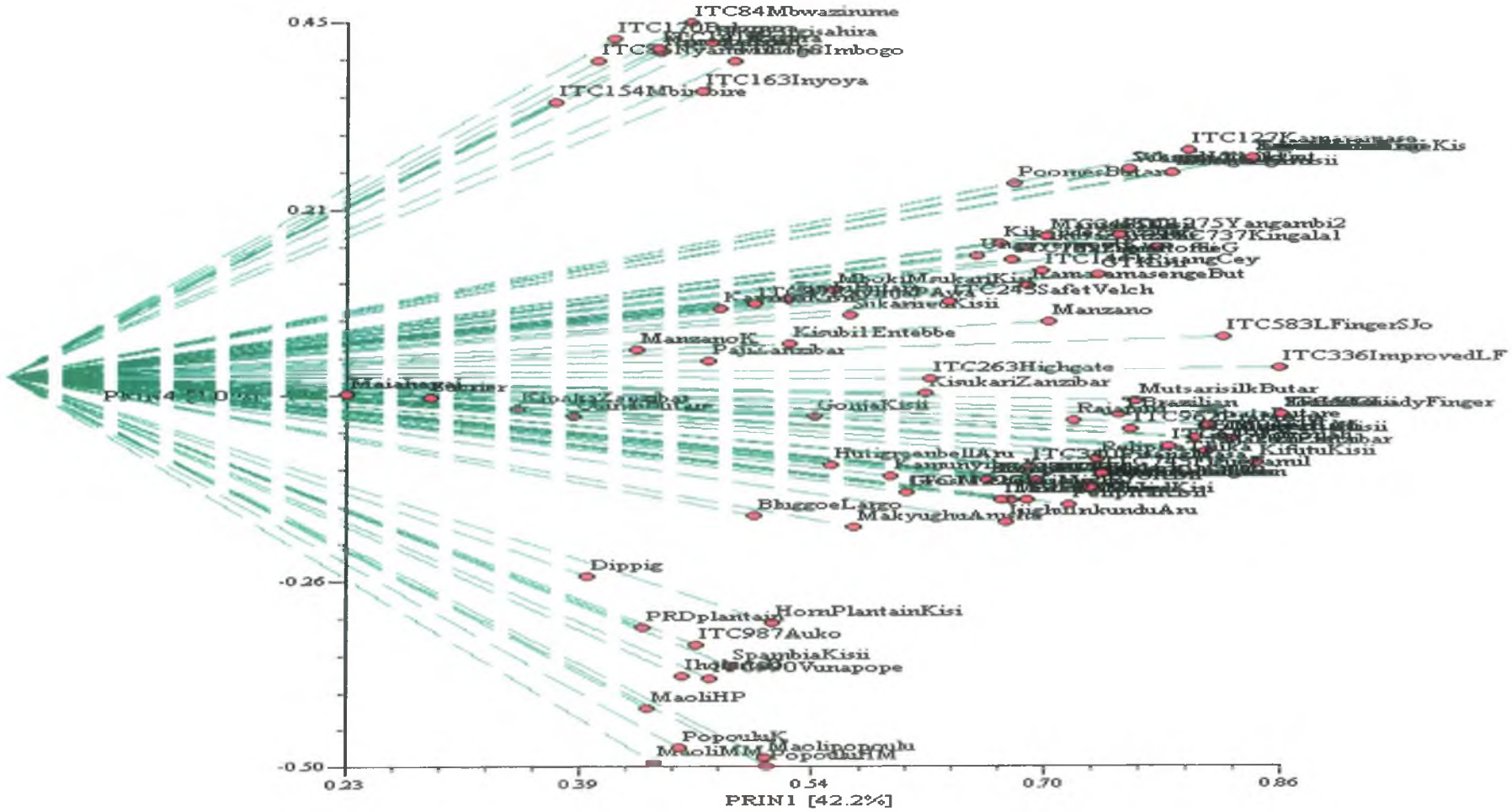


Figure 4. 6: PCA showing relative positions on the 1st and 3rd principal axes of 103 cultivated banana accessions, AAB "Apple" and AA "Muraru" and selected cultivated outgroups. Original variables superimposed on the plot as vectors

4.4. Discussion

The SSR markers revealed a high level of genetic variability among the AAB "Apple" banana taxa. It was not surprising that the highest mean similarities were those between the Prata and FHIA01, followed by that between "Sukari Ndizi" and GT as these are tetraploid hybrids of these natural cultivars. The ABB Kayinja and AB Kisubi cultivars, classified subjectively as juice or beer bananas also had a relatively high similarity (0.600). In addition, the dessert cultivars, namely, the Cavendish subgroup, Gros Michel, Prata, and "Sukari Ndizi" all had high similarities (>0.500) (Jaccard's). Overall the results supported the idea that genetic composition was highly correlated with the banana use. The plantains AAB and East African highland bananas AAA had, relatively, the lowest similarity < 0.400 with other sub-groups. In East Africa, plantains are subjectively classified as "roasting" and the EAH bananas as "cooking" types; in this study, there was relatively low similarity between the two types. All the phenograms produced showed overall similar topology and clustering patterns.

The clustering patterns formed with cultivars can be used to infer genetic relationships. From this study, the AAB "Apple" dessert bananas of East Africa formed four sub-clusters that were distinctively separate from the AB bananas. While these small-fruited AAB "Apple" bananas had generally been labelled as Silk, Lady Finger, or sometimes as Apple bananas, the four subclusters mentioned above also have distinct morphological characteristics (Chapter 3). The variability within the AAB "Apple" bananas had previously been thought to be limited. For example, using RAPDs, Uma *et al.* (2004) when evaluating the genetic diversity of some AAB Silk

bananas found narrow variability irrespective of geographical distribution. In this study, however, the data show that there was variability within the AAB "Apple" dessert bananas. Also, although all of the four taxa within AAB Apple were found in East Africa, the predominant and preferred type was the "Sukari Ndizi" taxon. This study also showed that the "Sukari Ndizi" was a distinct taxon having a number of accessions. The "Sukari Ndizi" comes in various synonyms; some are distinct somatic variants as shown in the cluster analysis. Although the small-fruited AAB "Apple" bananas are cultivated in many countries, (Simmonds and Shepherds 1955), each of the four taxa corresponds to a specific geographical region. For example, Prata is common in Brazil (Creste *et al.*, 2003, 2004), while the "Sukari Ndizi" or "Kamaramasenge" is prevalent in East African region.

The AA banana accessions such as ITC0249 Calcutta used in the breeding and banana improvement show only a relative similarity of about 0.2 with commercial AAA dessert, AAA cooking and AAB plantains. The current observation that AA "Muraru" has high relative similarity to these popular commercial dessert bananas suggests that they could be used in banana improvement. Another reason to consider the "Muraru" for breeding was their resistance to Panama disease.

In this study, it was also possible to separate some distinct accessions with the SSR markers that had not been previous separated by use of SSR markers because of the use of capillary gel electrophoresis that was able to detect small differences in fragment sizes making it feasible to separate some genotypes that could not be separated with the ordinary gel electrophoresis method.

Some of the East African "Apple" bananas, like Soth, Exera, Kifutu, Kijakazi, and Manyatta, clustered with AAB Prata, indicating a close relationship and similar genetic makeup. These were also corroborated by the morphological study, showing that these cultivars had the salient features of true Prata. However, there seemed to be some divergence within the Prata accessions, with the East African splitting from the Brazilian; only Kijakazi from Zanzibar was included in the smaller cluster with Prata and Prata ana.

In this study, accessions from East Africa; Manjano, Ungoye sweet, Mboki Musikari, and Pukusa clustered with the Silk cultivars from Bioversity International implying these cultivars are AAB Silk. Flow cytometry study confirms the genome group of these accessions. Accessions ITC348 Silk, ITC1222 Sports of silk, and ITC769 Figue pomme geante also in AAB Silk taxon was in agreement with the fact that they shared the *acuminata* genome from the sub-species *malaccensis* (Carreel *et al.* 2002). In this study, ITC769 Figue Pomme Geante clustered separate from Prata although they had similar chloroplast pattern (Carreel *et al.* 2002). From Carreel *et al.* (2002) study their mitochondrial types were different. Accession ITC348 Silk, ITC1222 Sports of Silk, ITC769 Figue pomme geante clustered with AB ITC245 Safet Velchi and Kisubi probably because of shared mitochondrial type δ (Carreel *et al.* 2002). Bhat and Jarret (1995) indicated that it is difficult to separate AAB and ABB genomic groups.

This study showed that SSR markers were very accurate and can be used in the identification of clones when there is a reference clone to be used.

The separation of accessions by PCA based on molecular SSR markers agreed with the subjective classification of usage an indication that there was a genetic component within their genetic makeup that makes a banana cultivar preferred for certain use. The separation was regardless of their genomic groupings of AAA or AAB. Simmonds (1959) noted that the subjective classification based on use may not be important in the classification of bananas by pointing out "cooking is a matter custom rather than necessity", and does not define any botanical meaningful class. In this study, the molecular classification seemed to agree with the subjective classification. The AAA cooking and AAB Plantain cultivars were separated by the larger PC from the AAA and AAB dessert banana accessions probably an indication that the separation on the basis of use has a genetic basis to it.

CHAPTER 5

ANALYSIS OF GENETIC DIVERSITY AND RELATIONSHIPS IN EAST AFRICAN ACCESSIONS OF *MUSA* AAB "APPLE" AND AA "MURARU" DESSERT BANANAS USING CHLOROPLAST MICROSATELLITE MARKERS

5.0. Introduction to chloroplast microsatellite use in banana classification

Although vegetative propagation of bananas allows the preservation of genotypes arising from crosses that occurred hundreds of years ago, the different cultivated clones of banana and plantain include hybrids of diverse composition, i.e., AB, AAB, AAAB, ABB, ABBB, AA, AAA, and AAAA genomic groups (Simmonds and Shephards 1955) and highly variable nucleus genotype, as demonstrated in Chapter 4. In contrast, chloroplast DNA is maternally inherited without meiotic recombination, and the multiple non-recombinant loci of chloroplast genomes give profiles that show exact genetic matches to those of the maternal parent, rather than the genetically variable populations arising from multiple heterozygous loci during gamete formation, as is the case for nuclear microsatellites. Chloroplast DNA microsatellite variation has been used to classify banana germplasm (Carreel *et al.* 2002). However, chloroplast microsatellites provide data on only one parent and, on their own, might not be variable enough to distinguish among possible parental assignments. Chloroplast haplotype in all populations may in some cases differ by only a single nucleotide.

5.1 Specific objectives of this study

This study of chloroplast microsatellite markers includes the following objectives:

1. To determine the capacity of universal chloroplast SSR primers to reveal polymorphisms and discriminate among banana accessions;
2. To group the various banana accessions based on common maternal descent as determined by the chloroplast inheritance; and
3. To concatenate the chloroplast and nuclear microsatellite markers' data into a single data set in order to provide an accurate classification of the study banana accessions.

5.2. Molecular analysis using chloroplast microsatellite markers

5.2.1. Polymerase Chain Reaction (PCR) and agarose gel electrophoresis

The same DNA samples used for the nuclear microsatellite analyses in section 4.2.3 were used in this study. Plant chloroplast 'universal SSR primers' (Cheng *et al.* 2006) were used in PCR amplifications of 133 bananas accessions. All reactions were carried out using Applied Biosystems Gene Amp® PCR system 2700 Thermal Cycler, programmed to perform 32 cycles. The reaction samples of 25 µl contained 1µl of 10 ng mL^{-1} genomic DNA, 12.5 µl Amplitaq (Applied Biosystems), 1.5 µl $MgCl_2$, 9.5 µl of filtered distilled de-ionized water, and 0.25 µl of each primer. The following PCR thermal protocol (developed earlier) (Cheng *et al.* 2006) was used: An initial denaturation step at 94^0C for 3minutes, was followed by 32 cycles of denaturation at 94^0C for 1 minute, annealing at 55^0C (or 59^0C for ARCP4) for 40 seconds, and extension at 72^0C for 1 minute, with a final extension at 72^0C for 5 minutes.

To check for amplification, 10µl fraction of the amplified product was separated on 2% agarose gel containing 5µl ethidium bromide run in 0.5xTBE buffer at 100v for approximately 2 hours and photographed using UV illumination. A total of 15 primer pairs were screened; 12 that amplified products with the bananas were selected and tagged with fluorophore for subsequent evaluation. The 12 fluorophore tagged primers were used in PCR reactions with all 133 banana accessions genotyped. Only 4 of the 12 primers produced polymorphic fragments, and these 4 primers (NTCP9, CCMP2, CCMP5 and ARCP4 (Table 5.1)) were used in this study.

5.2.2. Capillary gel electrophoresis

Fragment size detection of the PCR products using the fluorophore tagged primers (Applied Biosystems ABI) was done by capillary gel electrophoresis at the Centre for Genomics, Proteomics, and Bioinformatics Research Initiative (CGPBRI), University of Hawai'i at Manoa.

5.2.3. Data analysis

To visualize the alleles, the data obtained from the capillary gel electrophoresis were analyzed using Genemarker® 2004, version 1.51 (Soft Genetics LLC® State college, PA.). Figures 2a and b showed various allele sizes for the CCMP5 and NTCP9 primer pairs of 11 banana accessions. These allele calls were identified for each accession: presence was given a score of 1, while a score of 0 was given for the absence of an allele; only polymorphic alleles were scored. An allele was considered

polymorphic if it was present in at least one genotype and absent in the others. The data were then subjected to PCA using NTSYS PC, version 2.2.

Table 5. 1: SSR primer, fluorophore tag, annealing temperature Tm (^{0}C), number of alleles per locus, and fragment sizes

Primer #	**Tm (^{0}C)**	**Fluorophore tag**	**Number of alleles**	**Fragment sizes bp**
NTCP9	55	FAM	3	251, 259, 298
CCMP5	55	VIC	3	109, 114, 235
CCMP2	55	VIC	3	207, 230, 237
ARCP4	59	FAM	2	202, 203

5.3. Results

Figures 5.1 and 5.2 are examples of results of the allele reports for chloroplast DNA SSR markers from genotyping. The allelic reports for each clone show only one allele at any locus, since chloroplasts containing only a single circular chromosome.

Musa balbisiana ITC0094 and *Musa acuminata* from Lyon Arboretum, Hawaii, were separated from the rest of the 131 banana accessions in the study on the basis of chloroplast microsatellite variation at the four loci. Seven accessions, i.e., AAB Kluai Roi Wi ITC 0535, AAB Ney Poovan ITC 0459, ABB Kayinja-Kisii, ABB Kayinja ITC0087, *M. balbisiana* (Lyon Arboretum, Hawaii), Dippig, and Cameroun ITC0246, were grouped together by the four chloroplast microsatellite markers (shown as seven accessions with a B genome in Figure 5.3). The four chloroplast SSR markers were unable to split the remaining 124 accessions. These accessions were grouped as one (shown as 124 accessions in Figure 5.3) on the basis of chloroplast allelic variation or maternal decent.

Using both chloroplast and nuclear microsatellite markers, it was possible to distinguish the various banana groups as shown in Figures 5.4 and 5.5 displaying the phenograms for all the accessions.

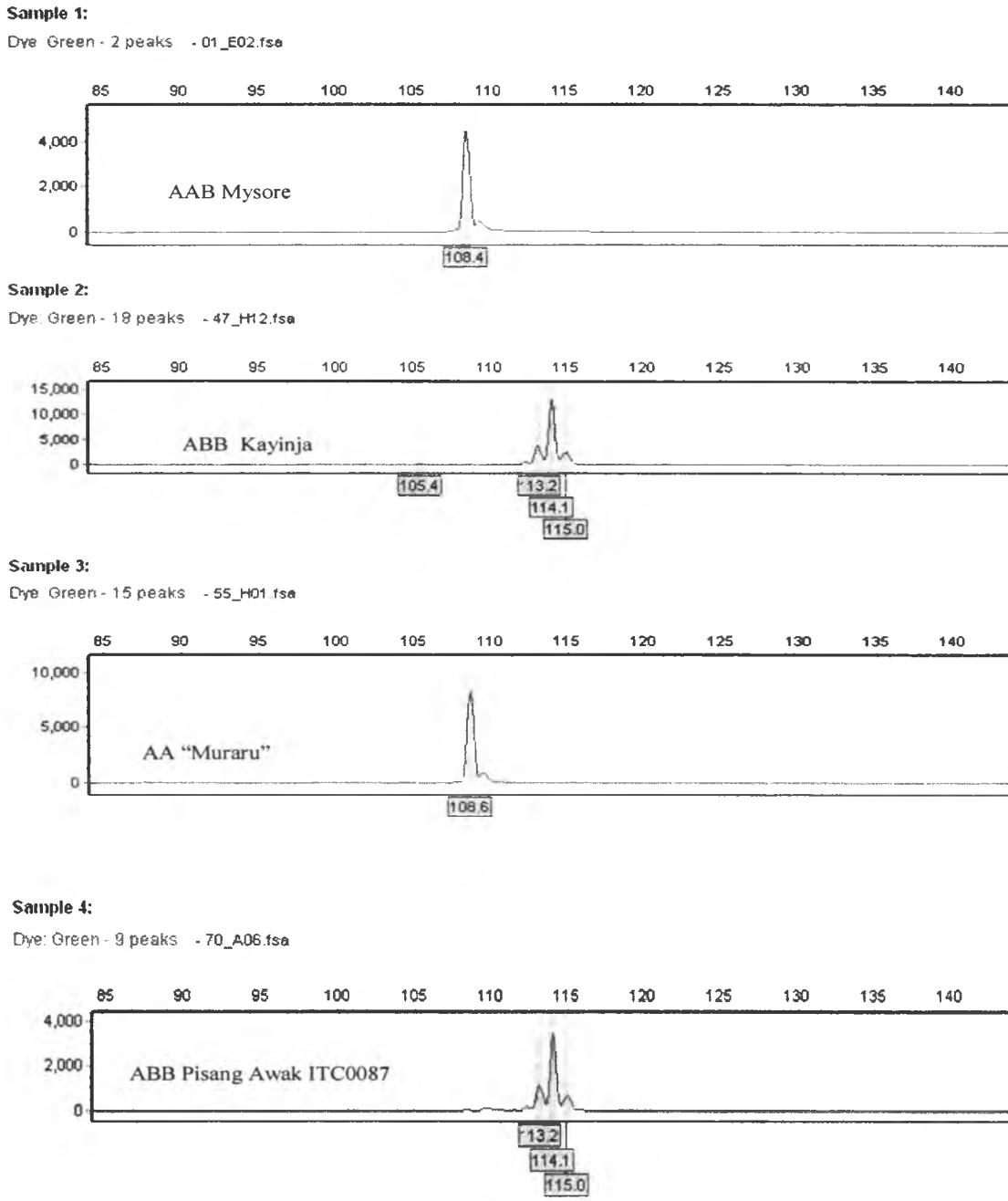


Figure 5. 1: Allele report of chloroplast SSR primer CCMP5 for four representative banana accessions. Each x-axis shows fragment size in basepair and y-axis show height of fragment peak. Each accession has only one allele at a locus.

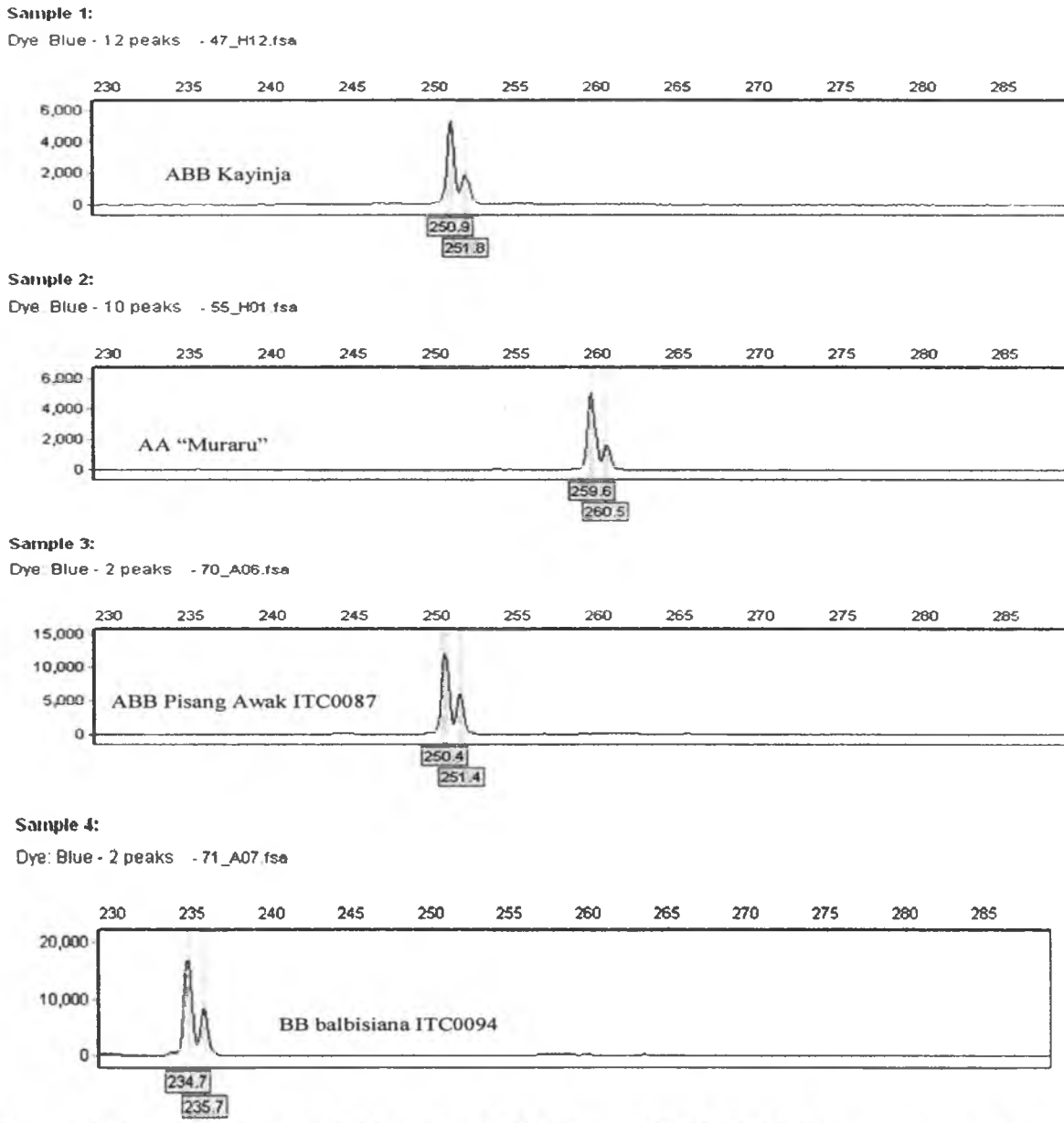


Figure 5. 2: Allele report of chloroplast SSR primer NTPCP9 for four representative banana accessions. Each x-axis shows fragment size in basepair and y-axis show height of fragment peak. Each accession has only one allele at a locus.

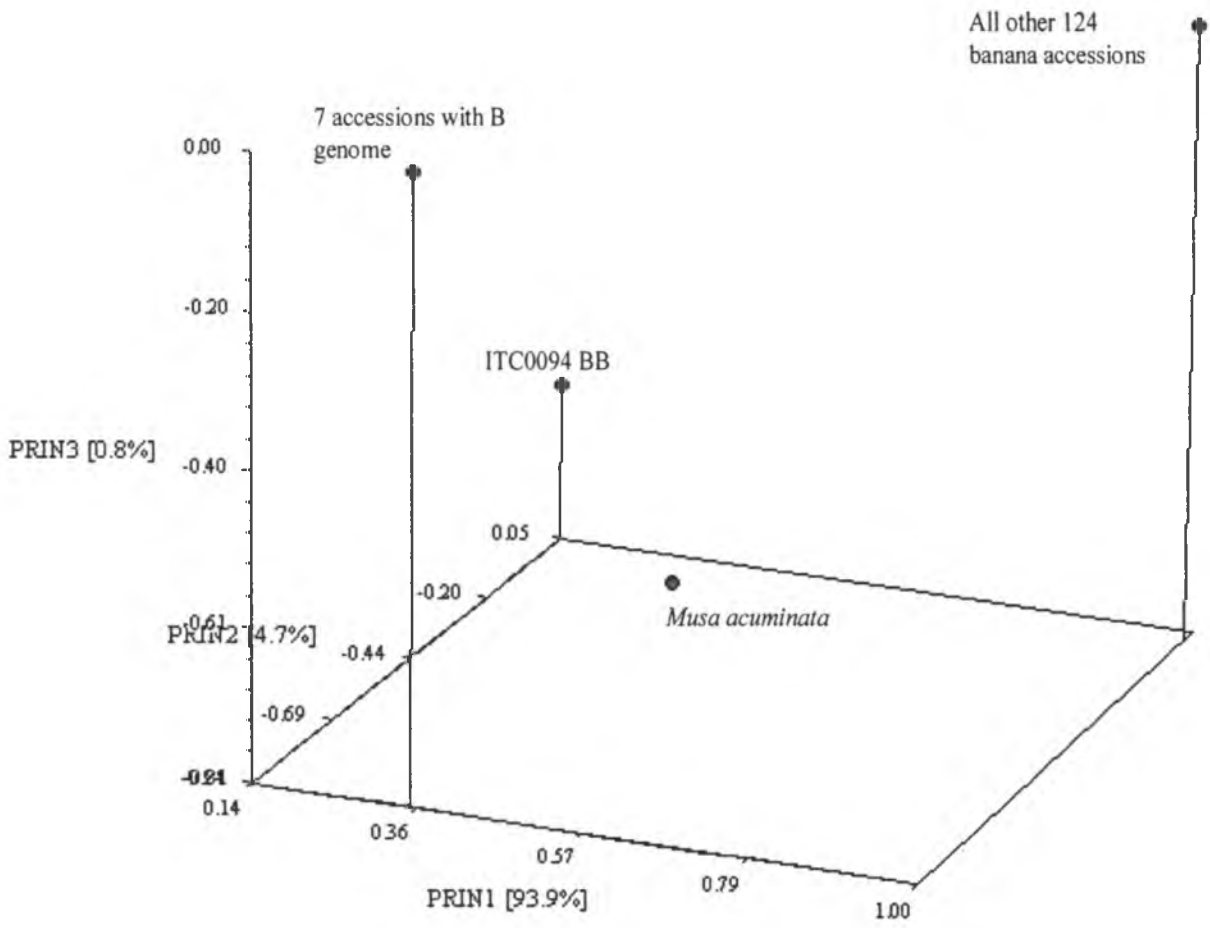


Figure 5. 3: PCA showing relative positions of 133 accessions, AAB "Apple" and AA "Muraru" and outgroups on the 1st , 2nd , and 3rd principal axes based on Chloroplast SSR marker variations. The figure depicts four different origins of maternally inherited cytoplasm that differentiate the banana accessions.

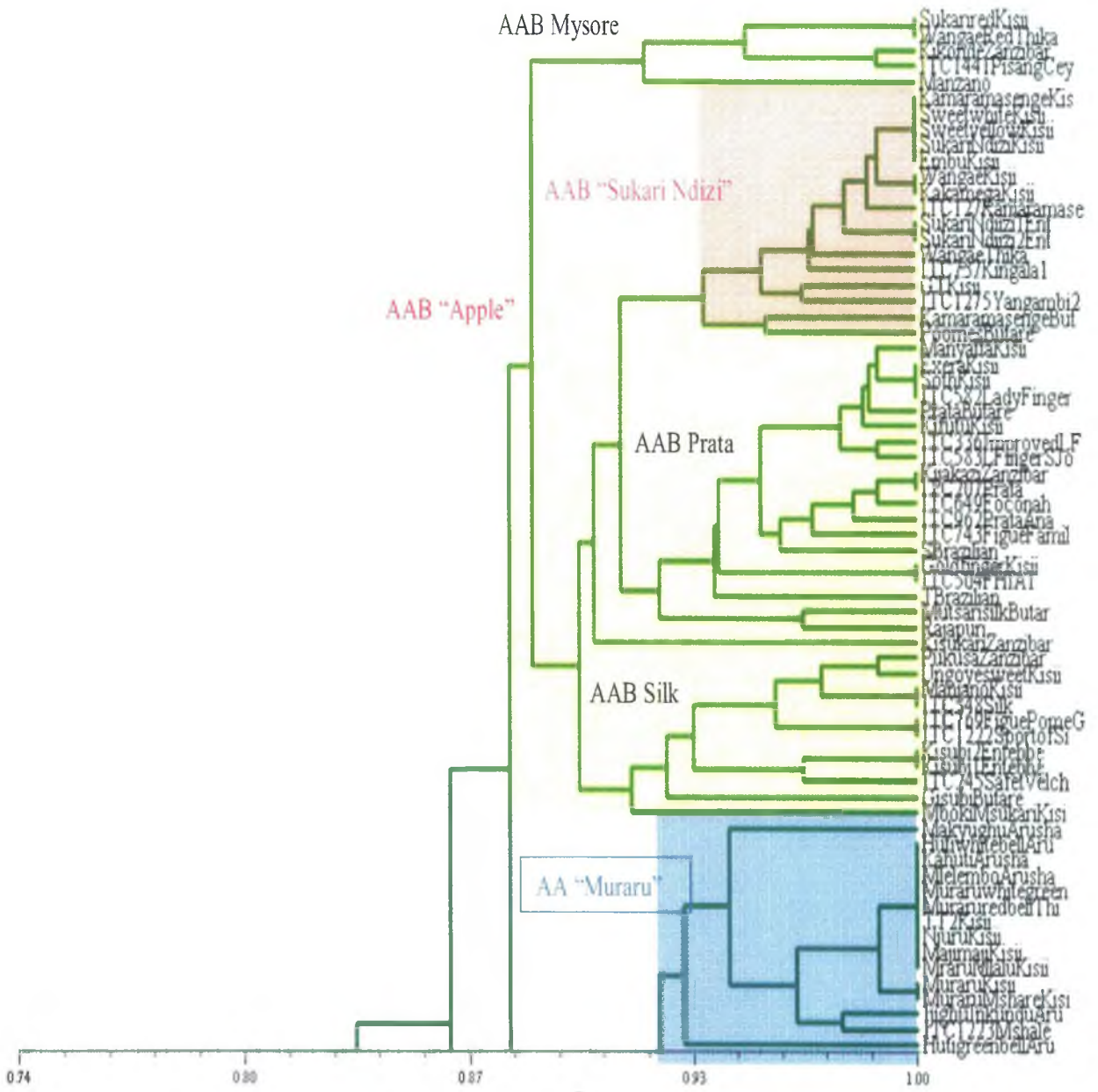


Figure 5. 4: Phenogram from UPGMA clustering using Simple Matching similarity coefficient among 133 banana accessions based on both nuclei and chloroplast microsatellite markers.
Cophenetic value = 0.897.

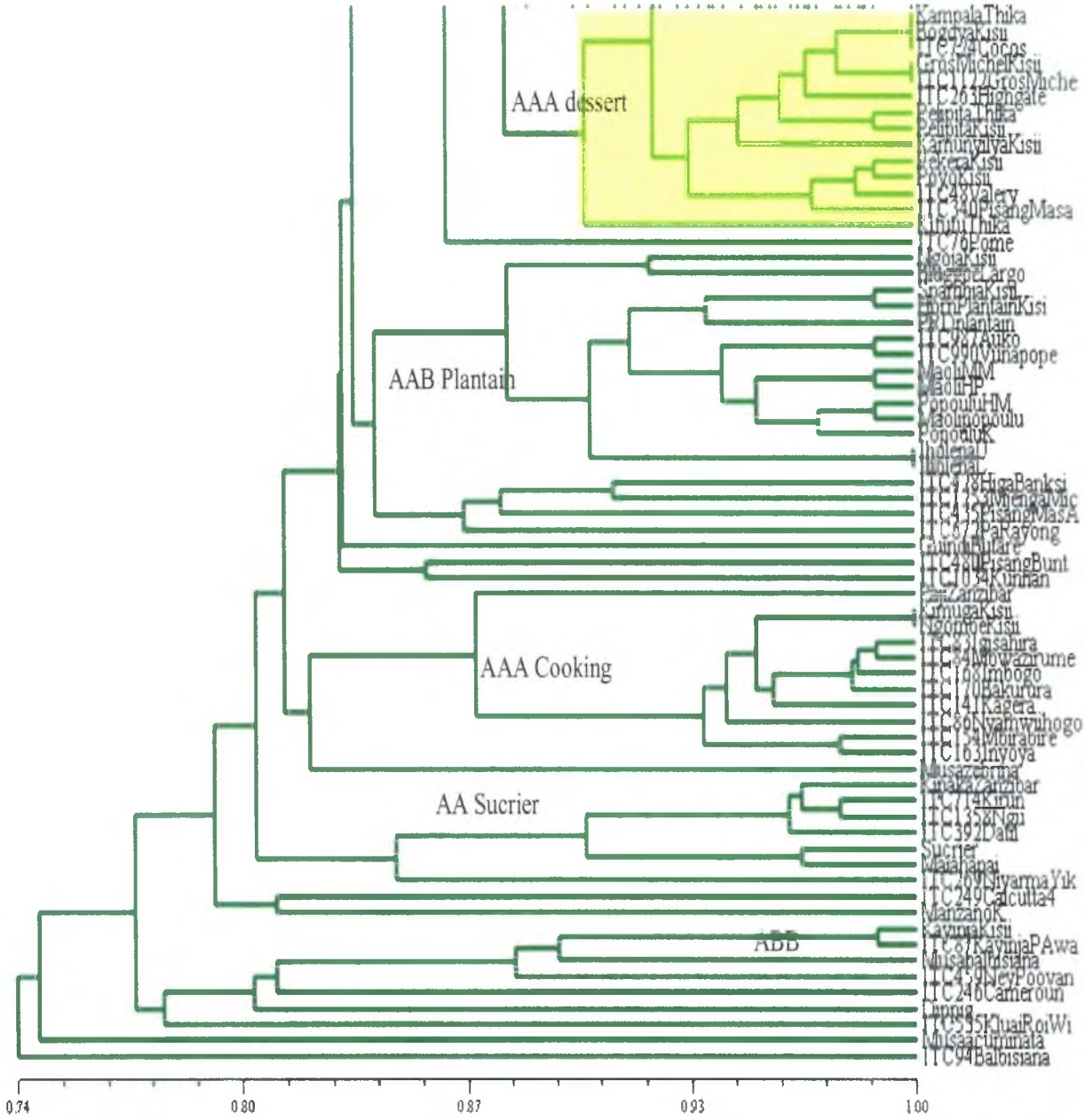


Figure 5.4: Phenogram from UPGMA clustering using Simple Matching similarity coefficient among 133 banana accessions based on both nuclei and chloroplast microsatellite markers.
Cophenetic value = 0.897.

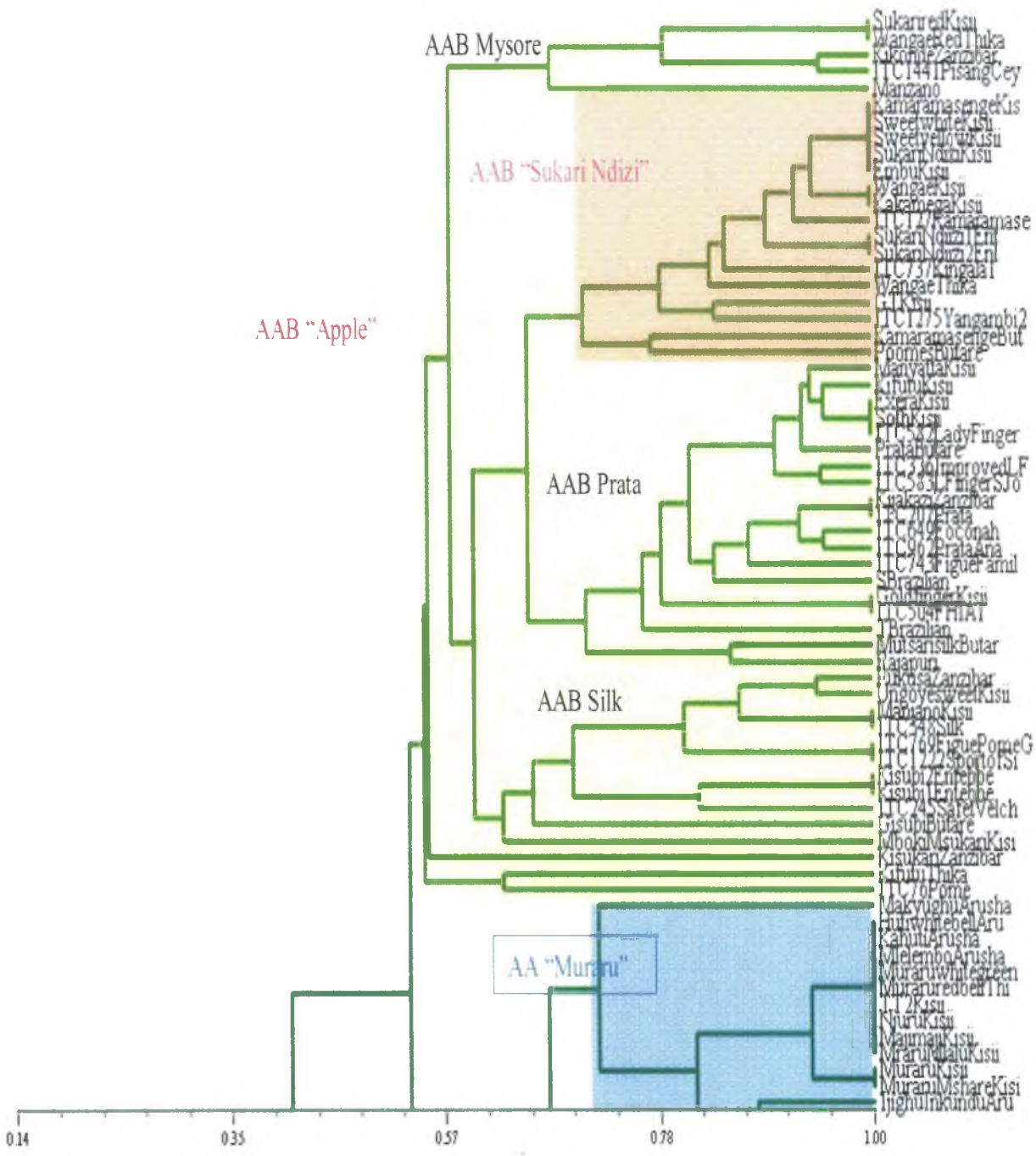


Figure 5.5: Phenogram from UPGMA clustering using Jaccard's similarity coefficient among 133 banana accessions based on both nuclei and chloroplast microsatellite markers.
Cophenetic value = 0.905.

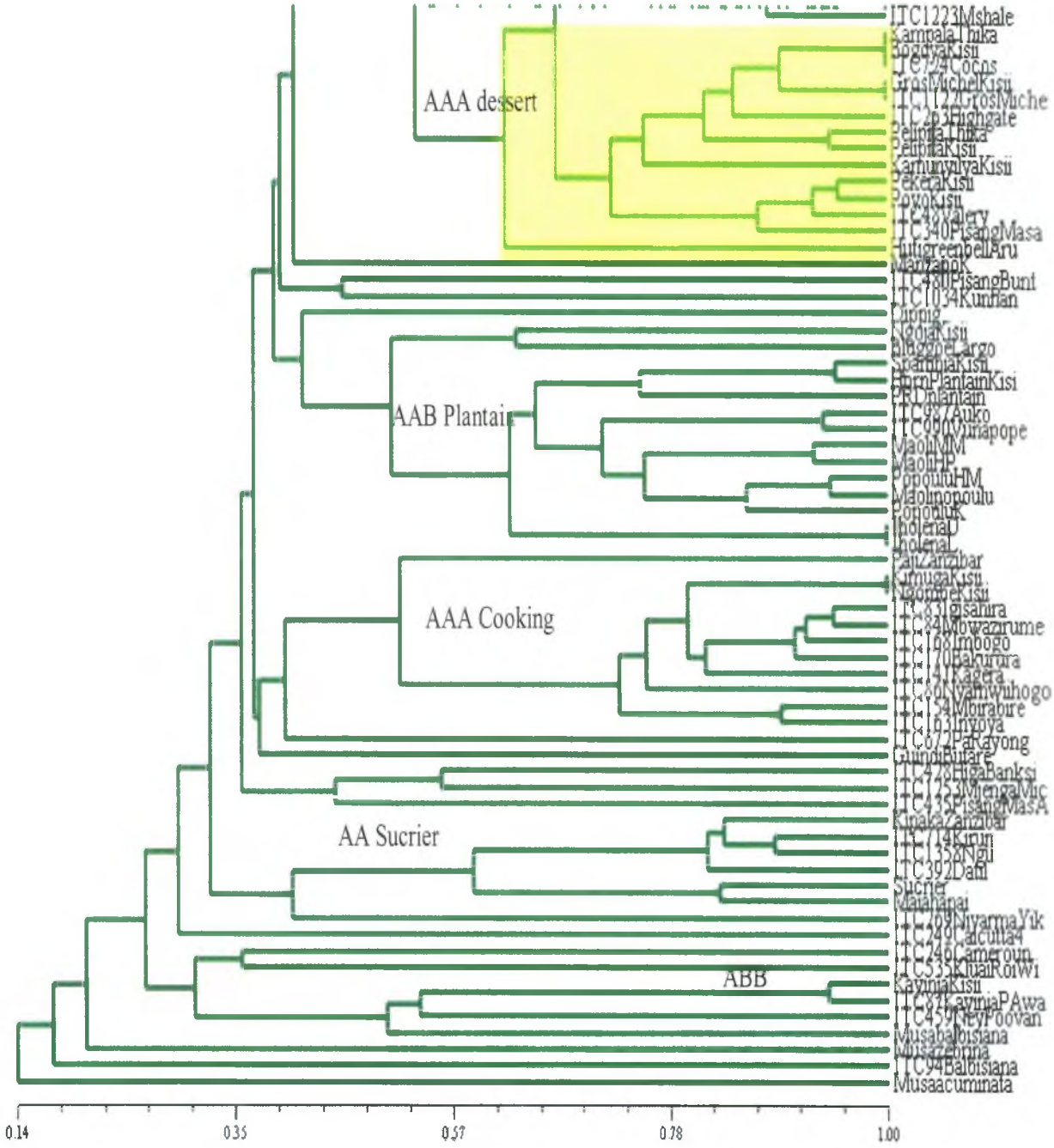


Figure 5.5: Phenogram from UPGMA clustering using Jaccard's similarity coefficient among 133 banana accessions based on both nuclei and chloroplast microsatellite markers.
Cophenetic value = 0.905.

5.4. Discussion

Chloroplasts are inherited maternally (Carreel *et al.* 2002, Faure *et al.* 1994). In this study, *M. balbisiana* ITC0094 and *M. acuminata* (Lyon Arboretum, Hawaii) each had a chloroplast pattern that was distinct from the rest of the banana accessions, indicating that they have unique maternal parents. Seven accessions (Kluai Roi Wi ITC 0535 (AAB), Ney Poovan ITC 0459 (AAB), P.Awak Kayinja ITC0087, Cameroun ITC0246, Kayinja-Kisii (ABB), Dippig and *M. balbisiana* (Lyon Arboretum, Hawaii)) probably share an ancestral *M. balbisiana* maternal parent, because two of these accessions only have the B genome. The remaining 124 accessions probably share an *M. acuminata* ancestral maternal parent, because many of them have genotypes consisiting of only the A genome. In this study, the plantains, Cavendish, Gros Michel, Muraru, East African cooking, and the AAB "Apple" accessions all have a similar chloroplast pattern. However, Carreel *et al.* (2002) using Restriction Fragment Length Polymorphism (RFLP), were able to separate the plantains and the East African cooking bananas from Cavendish, Gros Michel and "Apple' groups further to chloroplast pattern V, showing a closer relationship between the Cavendish, Gros Michel, "Muraru," and the "Apple" group. In my study using nuclei SSR markers, these same groups were shown to be more closely related to one another than with the East African highland AAA cooking banana. The results from this study were in agreement with Carreel *et al.* (2002) that placed Pisang Kelat (Kluai Roi Wi) and Pisang Awak (Kayinja) in the same chloroplast pattern VIII, which they shared with the three M. *balbisiana* types. In a separate substudy mentioned in section 3.2.3, also using nuclei SSR markers, Kluai Roi Wi clustered

closest to the *balbisiana* accessions Cameroun and ITC0094. Based on these results, the accessions collected from East Africa, including "Apple" and "Muraru" accessions all have a similar ancestral maternal parent, except for Kayinja-Kisii which shares an ancestral maternal parent with the Cameroun (BB), indicating that the ancestral maternal parent was of *M. balbisiana* origin.

The 124 accessions studied (over 93% of the total), including those from Bioversity International, Polynesia, and East Africa, had *acuminata* ancestral maternal parents. Although many (57%) of the accessions in this larger group of 124 accessions also have *balbisiana* genome in them, these all have an *acuminata* ancestral maternal parent. This is probably because although crossing is easy in both directions for the two *Musa* species (Simmonds 1954, 1962), hybrids with *balbisiana* maternal parents are weak and probably never survived under initial natural selection. As a result most hybrids of the two species have *acuminata* as maternal parent. This large group would probably separate to more chloroplast variant groups of *acuminata* origin if more markers were applied. Carreel *et al.* (2002) were able to separate sub-species Zebrina (pattern I) from the other *acuminata* sub-species (Errans, Burmannica Malaccensis, Microcarpa, Truncata (type II); Malaccensis and others (type III); and simaea (type IV). In their study Carreel *et al.* 2002 also separate two ABB Pisang Awak accessions based on chloroplast patterns (VIII and IX, although both had the same mitochondrial genotype ι). The particular set of four SSR chloroplast markers used here, did not separate the various bananan accessions of *acuminata* maternal parents further, and so it was difficult to say which of the sub-species might have contributed their chloroplast DNA. It was also possible that some of the various

acuminata sub-species could have the same chloroplast pattern, even with different genome since the chloroplast genome is slower evolving than nuclear DNA.

Carreel *et al.* (2002) found that many triploids have exactly the same chloroplast type as some diploid cultivars or wild clones. They concluded that these diploids are preferential parents and potential donors for breeding. In the their (Carreel *et al.* 2002) study, the sweet export bananas Cavendish, as well as the Gros Michel, Ambon, Rio, and Ibota, had the same cytoplasmic pattern as some 12 AA banana cultivars. This was also true for some seven AA cultivars, the AAA Red clones, the AAB Figue Pomme, and the AAB Silk clones. From the same study, Carreel *et al.* (2002) found that the plantains studied had exactly the same chloroplast pattern as some *M. acuminata* ssp. *banksii* accessions that are still present in Papua New Guinea indicating; few mutations of the cytoplasmic DNA might be expected.

The chloroplast genome is conserved in most respects. The order and arrangement of the chloroplast genes are nearly unchanging in most land plants (Hillis *et al.* 1996), and most variants that occur originate from one or a few simple inversions. Many cases of intra-specific chloroplast DNA variation do not appear to have risen in situ, but, instead, appear to result from chloroplast DNA introgression (Rieseberg and Soltis, 1991). In the case of the accessions that have both the *balbisiana* and *acuminata* constitution, the chloroplast probably originated from the original ancestral maternal parent by introgression during the early days, before selection for non-seedy character.

CHAPTER 6

ASSESSING NUCLEAR DNA CONTENT OF EAST AFRICAN *MUSA* AAB "APPLE" AND AA "MURARU" DESSERT BANANAS AND OTHER BANANA GROUPS USING FLOW CYTOMETRY

6.0. Introduction to ploidy level determination using flow cytometry

The study of cell biology has been used for the purpose of classification of plants (Lysak *et al.* 1999, Roux *et al.* 2003, Bartos *et al.* 2005). The cell is a basic unit of life, within which all of the genetic material is found and wherein all functions are initiated. Knowledge of the amount of genetic material contained in the cell is informative and opens a vast array of applications ranging from basic research to breeding (Dolezel and Bartos 2005). In this study, flow cytometry was employed to measure the DNA content within the cells of each accession; this was used to calculate the ploidy level.

In the terminology of flow cytometry, the phrase "genome size" is used to mean the DNA content of the monoploid genome or chromosome set, and 'DNA C-value" denotes the DNA content of the whole chromosome complement, or karyotype, without taking into account the degree of generative polyploidy of the organism (Greilhuber *et al.* 2005). Flow cytometry measures the content of nuclear DNA, based on the intensity of fluorescence produced by DNA-specific cytological stains. The ploidy level can be determined by comparing test samples with standards of known ploidy.

Flow cytometry determination of plant ploidy level was used because it is a rapid, accurate, convenient, and sensitive method compared to other conventional methods. Modern flow cytometers can analyze several thousand particles every second, in "real time," and can actively separate out and isolate particles having specified properties. Propidium iodide (PI) is used for staining nucleic acids, since it intercalates between base pairs of double-stranded DNA and RNA with little or no base specificity (Properi *et al.* 1991). PI is excited by visible light with an absorbance maximum at 490nm and its emission maximum is at 620nm.

Various workers have used the flow cytometry method in banana evaluation (Dolezel *et al.* 1994; 1997, Lysak *et al.* 1999, Kamate *et al.* 2001, INIBAP 2005) and some have found that the A and B genomes of *Musa* differ in size (Dolezel *et al.* 1994, Lysak *et al.* 1999). It was hypothesized that not only could flow cytometry be used to determine ploidy level, it was possible to use flow cytometry to distinguish the various banana genomic groups and identify unknown/uncertain banana accessions.

Since the size difference between *Musa* A and B genomes was small, it was important that very accurate measurements be taken to detect differences, especially when comparing accessions having the same ploidy levels but different genome compositions. Calculation of the amount of DNA in cell nuclei is based on the ratio of the sample nuclei to that of the standard nuclei of known DNA amount, stained with a DNA-intercalating dye. Since DNA content is directly proportional to the fluorescence intensity, the accurate determination of DNA content becomes a problem of precisely measuring the sample fluorescence relative to that of the

standard reference nuclei (Dolezel 1999). The pea *Pisum sativum* L. was recommended as a good standard for flow cytometry when using PI (Spencer *et al.* 1999), and Greilhuber and Ebert (1994) found that the garden pea plant (*Pisum sativum*) had a stable DNA content over geographically diverse collection and could be used for this purpose. For flow cytometry, it is preferable to select an internal standard with 2C and 4C peaks close to, but not overlapping, those for the target species, so that large errors are not introduced by relatively small random errors in the estimated mean of the standard itself (Spencer *et al.* 1999).

Dolezel (1999) further described use of pea as an internal standard in plant nuclei preparations for flow cytometric determination of DNA content. Pea leaf tissue samples were combined with each sample of the test species, and nuclei were co-extracted, so that minute variations in staining conditions from sample to sample would be negated by affecting the sample and reference standard equally (Doležel 1991).

To aid precision in this study, nuclei of both the *Pisum sativum* and test samples of *Musa* were isolated from their leaves and analyzed simultaneously. The ratio of fluorescence intensity of *Musa* and *Pisum sativum* G1 nuclei was used to calculate DNA content and ploidy. Dolezel *et al.* (1994) found the use of an internal standard suitable for detection of very small differences in DNA content, as low as 1.6 % between various *Musa* genotypes.

6.1. Specific objectives of the flow cytometry study

The following are the stated objectives of this portion of the study:

1. To determine the ploidy of the various accessions of East African "Apple" AAB and AA "Muraru" dessert banana groups plus outgroups; and
2. To use the DNA content to resolve the genome composition of the various accessions studied.

6.2. Materials and Methods

6.2.1. Study sample material

A total of 90 banana accessions in Table 6.2, a subset of those in Tables 4.1, 4.2, 4.3, and 4.4, were used in this study at University of Hawai'i at Manoa. These included accessions that were obtained locally in Hawaii, or from Bioversity International in Leuven, Belgium, or from collections of the Kenya Agricultural Research Institute at Kisii and Thika, Kenya, or from similar collection at Tengeru, Arusha Tanzania. Imported accessions were shipped in vitro after various indexing for viral diseases, according to state and federal import regulations. A further 39 accessions (Table 10) that were not possible to bring into Hawaii were analyzed at the International Livestock Research Institute (ILRI), Nairobi, Kenya.

6.2.2. Plant sample preparation for the flow cytometry analysis

An important constraint in the use of flow cytometry in plants is the requirement for single cell suspensions. The complex three-dimensional structures of plant tissue and the cell walls are generally incompatible with direct-flow cell cytometric analysis. Consequently, a standard method of extracting free cell nuclei from leaf tissue was employed. A razor blade was used to manually chop the leaves

in "extraction buffer" (Partec GmbH, Münster Germany), thereby releasing the cell nuclei into suspension, along with the remaining cellular organelles and a variety of other forms of debris (Galbraith *et al.* 1983). Large debris was then removed with the nylon filters; the whole process is explained in detail with the next steps below.

6.2.3. Nuclei isolation and fluorescent staining

A 50 mg sample of fresh leaf midrib of each banana accession, together with a 10mg sample of fresh leaf of pea (*Pisum sativum* L.) as internal standard, were chopped with a single razor blade to semi-fine fragments in a 5-cm plastic petri dish (Figure 6.1) containing 1000 µl of "extraction buffer" (Partec GmbH, Münster Germany). After 1 minute of incubation in the extraction buffer, the crude suspension of isolated nuclei was filtered through 50µm pore size nylon filters (Partec GmbH, Münster Germany). The filtrate was pelleted by centrifugation (300*g*, 5min) and resuspended in 200µl of extraction buffer, gently vortexed, and incubated for 1 hour at room temperature. Finally the filtrate was stained with 600 µl staining solution (600 µl of staining buffer with 3.6 µl of propidium iodide stock solution and 3.6 µl of RNAse stock solution). After 1hr the stained nuclei were analyzed by flow cytometry.

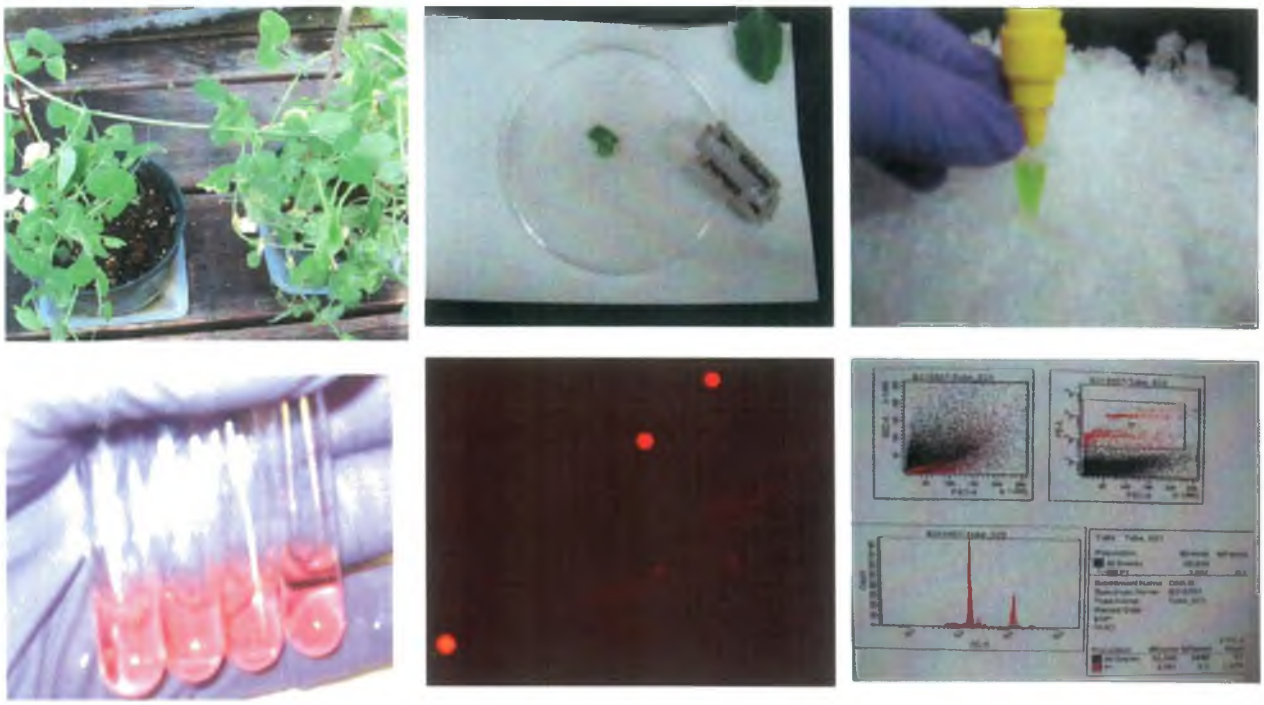


Figure 6. 1: Pictures showing protocol of flow cytometry as described in section 6.2.3. top left, *Pisum sativum* plant, leaves of banana and *P. sativum* in extraction buffer in petri dish ready for chopping, filtering the suspension. Bottom left, PI stained filtrate, PI stained cell nuclei seen under microscope, and computer output of flow cytometry results.

6.2.4. Flow cytometry analysis

The suspensions of propidium iodide-stained nuclei were analyzed by flow cytometry with a Beckman-Coulter (Miami, Florida) Altra flow cytometer at University of Hawai'i at Manoa (www.soest.hawaii.edu/sfcf) using the 488 nm line of a Coherent I90C argon ion laser set at 200 mW. The linear, log, and peak fluorescence signals of the propidium iodide-stained nuclei were collected (610 BP filter, 640 DCLP filter), along with forward and side scatter signals. Plots of peak vs. linear propidium iodide fluorescence were used to eliminate doublets. Histograms of

linear DNA fluorescence were analyzed in FlowJo (v. 6.3.4, Treestar Inc., www.flowjo.com).

Three plant samples were analyzed per accession or genotype. To avoid any bias due to instrument drift, leaves from each plant sample were measured on different days. Most of the banana nuclei had a single DNA peak; the pea standard had a single DNA peak. Each histogram represented more than 1,000 nuclei. A 2C nuclear DNA content, defined as the DNA present in the G1 phase of the cell cycle, was determined using the fluorescence intensity peak positions. Based on these intensity peak values, the nuclear DNA content of each accession was calculated (pg) in relation to the *Pisum sativum* 2C value of 9.07pg DNA. The formula used was:

Musa accession 2C DNA content of = *Musa* accession G1 fluorescence intensity peak mean X 9.07 (the *Pisum sativum* 2C value) ÷ the G1 fluorescence intensity peak mean of *Pisum sativum*.

The genome size or the DNA content of the monoploid genome was calculated by dividing the 2C nuclear DNA by ploidy. To convert the values in number of nucleotide (Mbp), the values obtained were multiplied by 0.965.

6.2.5. Statistics - data processing SAS program

The 2C nuclear DNA content data were analyzed using Statistical Analysis System (SAS) program (Cary, N.C). The general linear model (GLM) method was used. Since each accession had 3 replications of data from the 3 different days of sample analysis, the flow cytometry experiment was a replicated one in a randomized complete block design. The different days were treated as blocks, since it was

expected that probably the reagent mix (i.e., the PI proportion in the mix) would be slightly different during the different days, giving slightly different fluorescence results. This was minimized by the use of an internal standard. The data of the DNA content were subjected to ANOVA to determine if there were any significant differences between the accessions, and then Duncan's multiple range tests to establish the Duncan grouping. The same data were further subjected to ANOVA based on banana genome groups and then Duncan's multiple range tests to establish the Duncan grouping.

6.3. Results

The banana cell nucleus extraction procedure produced preparations useful for DNA content analysis by flow cytometry (Figure 6.1). Preparations were examined with an Olympus BX51 upright compound microscope equipped with a U-MWG2 Wide Green filter cube (510 – 550 nm excitation, 570 nm barrier filters, and 590nm long pass emission). Images were recorded with an Optronics Macrofire SP CCD camera.

Flow cytometry analysis generated histograms showing fluorescence-intensity frequency distributions of populations of banana nuclei relative to the standard reference nuclei of pea. Several examples comparing histograms of banana nuclei extracted from accessions with different genome compositions are shown in Figure 6.2 (linear fluorescence scale) and Figure 6.3 (log fluorescence scale). Figure 6.2 shows histograms of relative nuclear DNA content in linear scale of nuclei isolated from banana leaf of a diploid AA, triploid ABB, and triploid AAB; Figure 6.3 shows histograms of relative nuclear DNA content in log scale of nuclei isolated from

banana leaf of a triploid AAB, a triploid ABB, and a diploid AA. All the histograms on the right in each figure are of standard *Pisum sativum* nuclei.

The 2C DNA content and the monoploid genome size of all 90 banana accessions were calculated from the fluorescence data and analyzed using ANOVA. Tables 6.1a and 6.1b are the ANOVA results. Further grouping based on 2C DNA value and monoploid size of all the banana accessions using Duncan multiple range test are arranged in order of descending size in Tables 6.2 and 6.3, respectively.

With regard to Table 6.4, the descending order of the 2C DNA values clearly groups banana accessions by genomic composition, starting with the largest tetraploid clones at the top and ending with the smallest diploid *Musa balbisiana* accessions at the bottom. ANOVA indicated that this variation in 2C DNA content among accessions is highly significant ($p < 0.01$).

The Duncan's Multiple Range test showed that the ploidy level of banana accessions affected the 2C DNA content significantly; therefore, flow cytometry unambiguously revealed the number of genome copies in each accession (Table 6.4 and Figure 6.4).

While there was no meaningful difference between the two accessions at the tetraploid level, significant variations in 2C DNA content existed within the diploid and triploid levels.

Among diploids, the nuclear DNA content of *Musa balbisiana* (BB) accessions was significantly less than that of *Musa acuminata* (AA) accessions, although there was also significant variation within each of these diploid species. The

diploid AB had intermediate 2C DNA content significantly different from the neighboring *M. acuminata* or *M. balbisana* accessions (Figure 6.4).

Among triploids, a similar pattern of statistically significant distinctions was observed between genome groups (AAA, AAB, and ABB). All ABB accessions had significantly smaller 2C DNA contents than any of the AAA accessions, but AAB accessions were intermediate, and accessions in the lower part of the AAA nuclear DNA range were not significantly different from accessions in the upper part of the AAB nuclear DNA range. Similarly, accessions in the lower part of the AAB nuclear DNA range were not significantly different from accessions in the upper part of the ABB range. Again, 2C DNA variation in triploids taken collectively was continuous and decreasing towards genome compositions with more *M. balbisana*.

2C DNA content variation between accessions within the triploid AAB or ABB genomic groups was significant, but not within the AAA group.

Figure 6.4 is a graphical representation of the mean 2C DNA and the monoploid size (pg) of various banana genomic groups studied, and Figure 6.5 is the effect of the ploidy level on the monoploid size of the various banana genome groups.

The range in the 2C DNA between the AAA triploid and AAB was 8.3 to 6.5%, while only there was 1.9% difference in the 2C DNA within the genome group. Within the AAB, the difference in the 2C DNA was 5.5 % and significant. The largest difference in genome variation was found among the AA and was 8.9%. The 2C DNA difference between AA and BB genomes as a range of 13.8% to 17.5%, and the range between AB and BB genomes was 3.9% to 8.5%, while that between AA and AB was a range of 3.9% to 8.5%. Variation in 2C nuclear DNA content within

both the AA and BB genomes contribute to the observed differences within and between AAA and AAB genomic groups.

With regard to Table 6.3, ordering the 90 accessions on the basis of monoploid genome size from largest to smallest results in a gradient of clones with a generally decreasing proportion of the A genome and an increasing contribution from the B genome. Examples of exceptions include BB Cameroun, a *M. balbisiana* with an unusually large genome size, and AA Njuru, a *M. acuminata* with an unusually small genome size. Superimposed on this trend, and partly responsible for deviations from it, is another pattern that finds larger genome sizes in diploids and smaller genomes in higher ploidy groups. For example, the tetraploids AAAB GT and AAAB "Goldfinger" ought to be at the top of the "predominantly A" genome group but they fall more toward the middle, and diploid AB "Safet Velchi" ought to be at the bottom of the same group, but instead it appears near the top (Figure 6.5).

ANOVA indicated that the variation in monoploid genome size among accessions is highly significant ($p < 0.01$). The Duncan's Multiple Range test showed a continuum of genome size values with significant differences between accessions that are generally widely separated in Table 6.3 and a critical range of 0.0114.

In general, the diploid bananas, e.g., AA and AB had higher monoploid genome size compared to triploid AAA and AAB genome groups respectively (Figure 6.4 and Table 6.4). This trend is also reflected in the fact that the mean 2C DNA contents of triploid and tertraploid genotypes are smaller than the simple sum of monoploid A and/ or B genomes constituting the respective polyploidy groups. These results are consistent with the fact that higher ploidy genomes are able to tolerate the

loss of parts of their genome (since they have duplication of the same), while diploids are less tolerant to loss because they are normally lethal; diploids that survive have to have their complete genome. However the *balbisiana* genome was always significantly smaller than the *acuminata* genome.

Some accessions, namely, ITC0459 Ney Poovan, ITC 0990Vunapope, and ITC0987Auko documented as diploid AB (INIBAP), had 2C DNA values that strongly suggests they are triploid AAB. The flow cytometry and SSR analyses also suggest that Datil is an AA diploid and not AB, as documented (Bioversity International).

Table 6.4 shows the banana accessions' groupings based on their monoploid genome size, or the DNA content of a chromosome set done by Duncan's multiple range test. The values are in Mbp. The coefficient of variation for the genome size was low (2%) and the R-square high (87%); these are indications of data consistency and reliability. There were significant differences in monoploid genome size values of accessions having the A-only genome and those with B genomes. A few of the A-only genomic group accessions had significantly bigger monoploid genome sizes than other A-only genomes; the range was from 627 Mbp to 571 Mbp. The accessions with some B genome but with more A proportion, had monoploid genome sizes ranging from 569 to 544 Mbp, while those with B-only genome (or a higher proportion of the B genome) had monoploid genome size ranging between 544 to 517 Mbp, except for the Popoulu Ka'io (AAB) which is suppose to have more A. Could the Popoulu Ka'io be an ABB or is this just an overlap with ITC0246 Cameroun (544Mbp) having generally a bigger monoploid genome size as also found by Lysak

et al. (1999). There was also an overlap between Njuru AA and Iholena upehupehu AAB in monoploid genome size values (571 Mbp). There was a general trend of the A-only or the mainly A proportion accessions to have bigger monoploid genome size than the B-only or higher B proportion genome accessions (Table 6.4).

Table 6.5 is the average DNA content, obtained with the accessions analyzed at the ILRI laboratory in Nairobi, Kenya, using a different flow cytometer instrument. The data is compared separately to avoid variation that may result from other variables, apart from the test accessions.

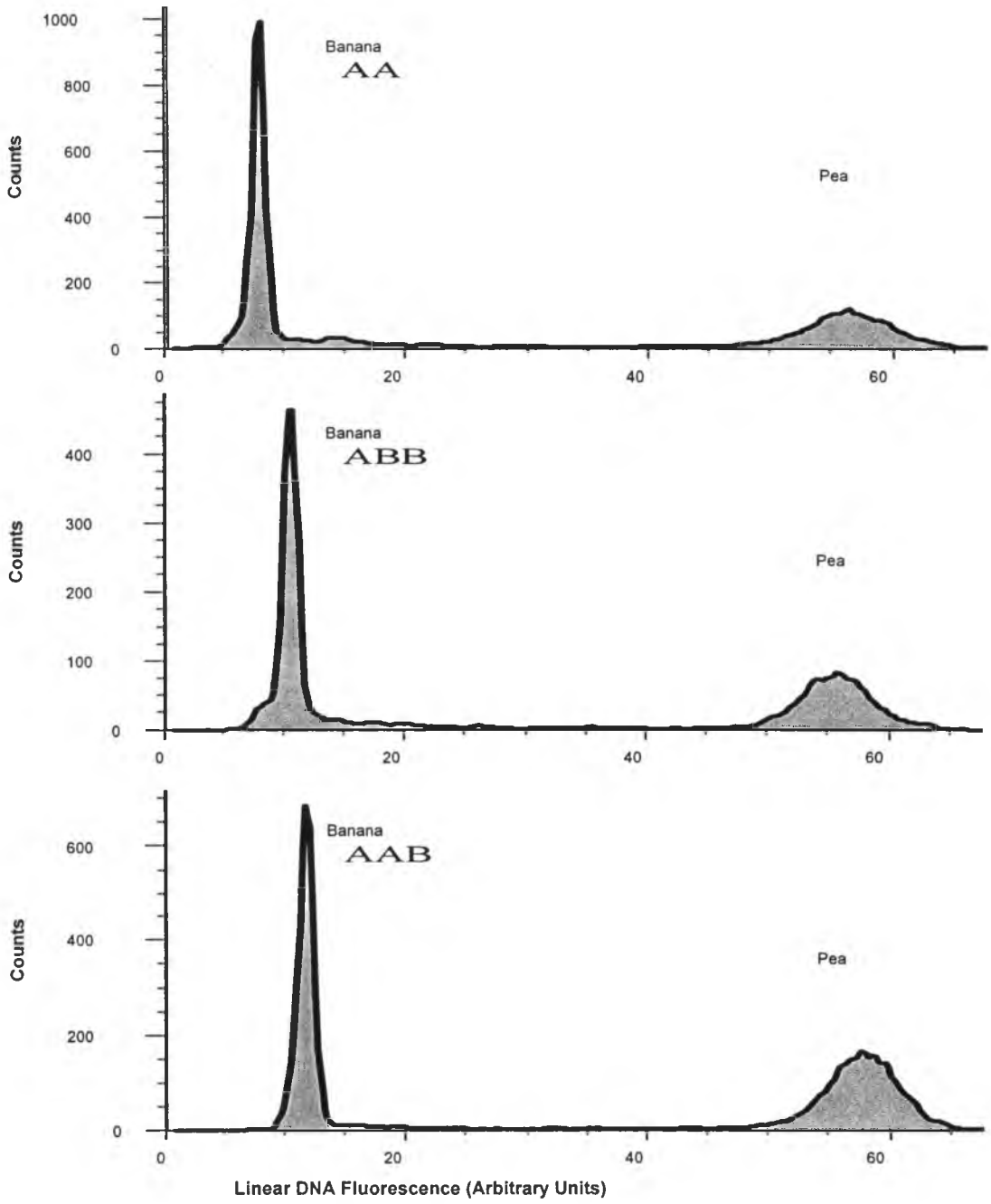


Figure 6. 2: Histograms of relative nuclear DNA content (linear scale) of nuclei isolated from the banana leaf of (top left) a diploid AA, (middle left) triploid ABB, and (bottom left) triploid AAB. All the histograms on the right are of standard *Pisum sativum* nuclei.

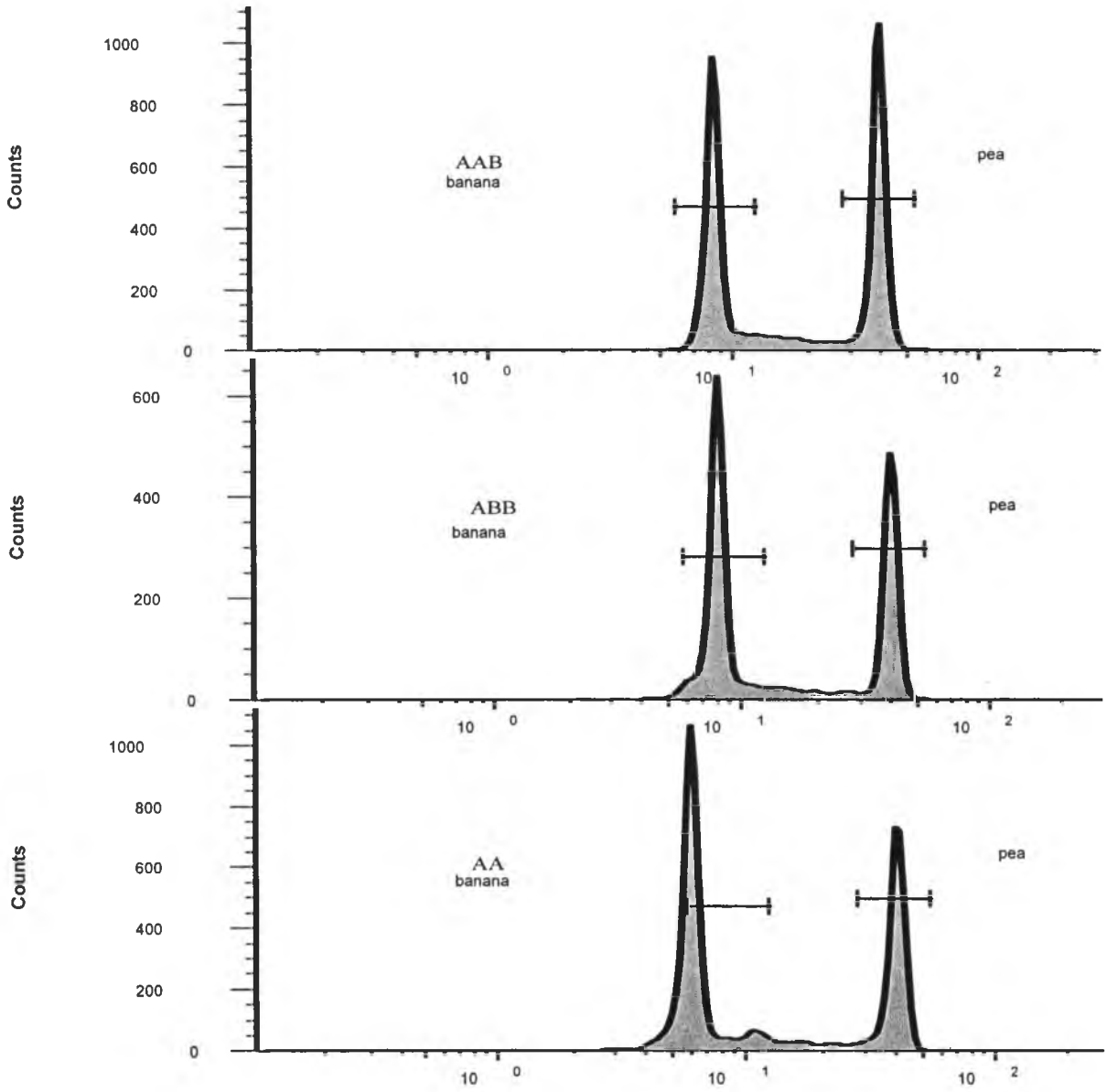


Figure 6. 3: Histograms of relative nuclear DNA content (log scale) of nuclei isolated from the banana leaf of (top left) a triploid AAB, (middle left) triploid ABB, and (bottom left) a diploid AA. All the histograms on the right are of standard *Pisum sativum* nuclei.

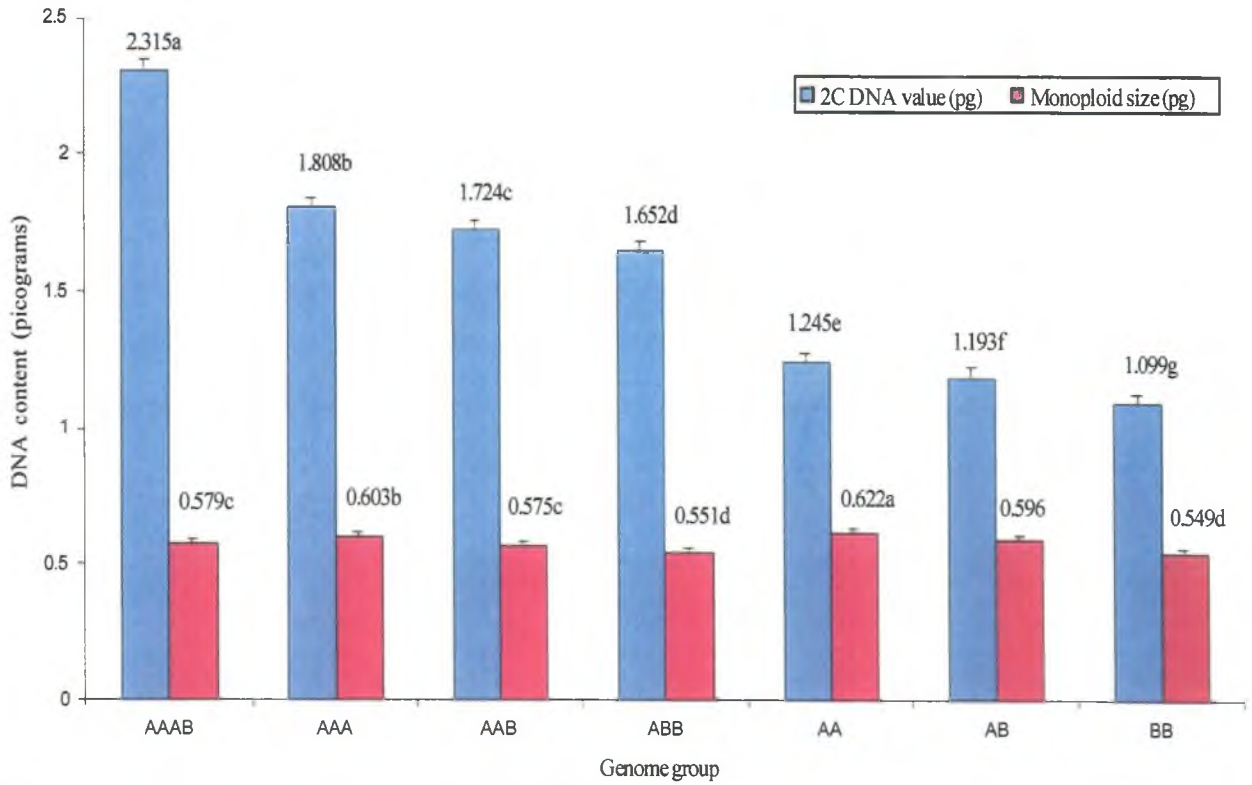


Figure 6. 4: Mean 2C DNA and Monoploid size (pg) of various banana genomic groups studied.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

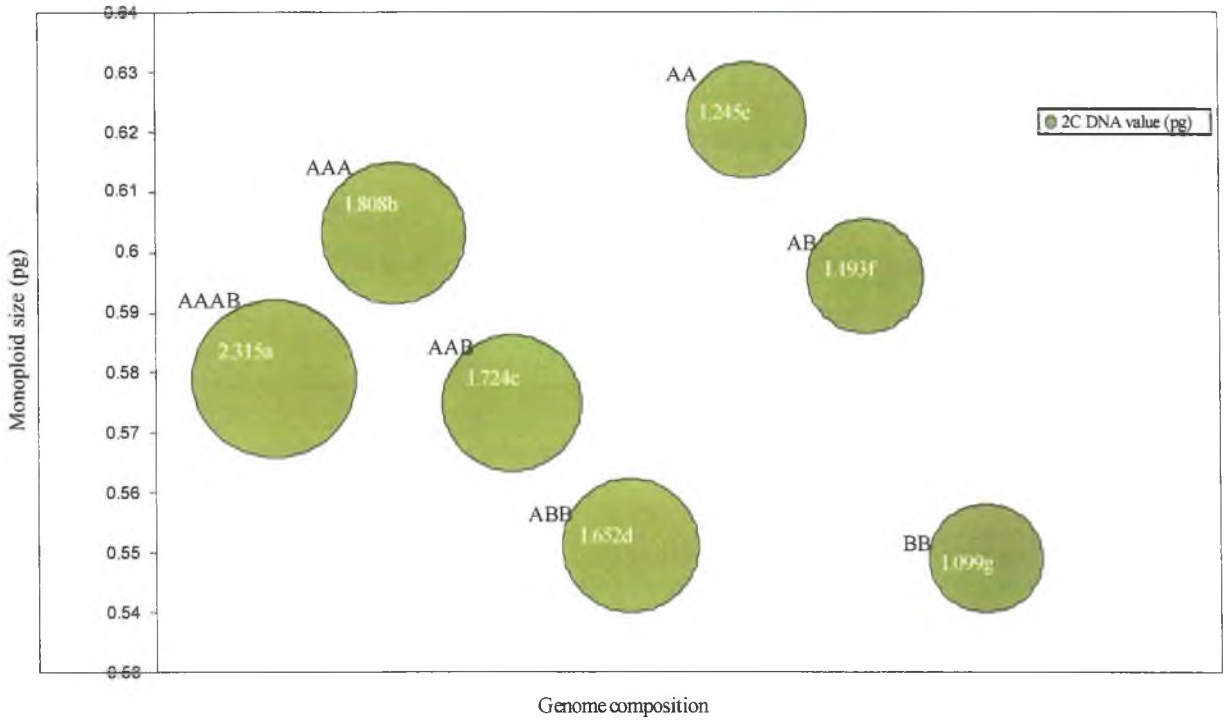


Figure 6. 5: The effect of Ploidy level on the Monoploid size of the various banana genome groups.
Values followed by the same letter/s coding are not significantly different among bubbles according to DMRT at $p \leq 0.05$.

Table 6. 1a: The GLM Procedure for 2C DNA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	18.12021478	0.19912324	238.06	<.0001
Error	178	0.14888423	0.00083643		
Corrected Total	269	18.26909901			

R-Square	Coeff Var	Root MSE	Lin_DNA Mean
0.991850	1.799727	0.028921	1.606970

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	2	0.02568483	0.01284241	15.35	<.0001
Genomic_group	6	17.96838969	2.99473161	3580.38	<.0001
accession(Genomic_g)	83	0.12614026	0.00151976	1.82	0.0005

Tests of Hypotheses Using the Type III MS for accession(Genomic_g) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genomic_group	6	17.96838969	2.99473161	1970.53	<.0001

Table 6.1b: The GLM Procedure for Monoploid size (pg)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	0.15775394	0.00173356	12.85	<.0001
Error	178	0.02401762	0.00013493		
Corrected Total	269	0.18177156			

R-Square	Coeff Var	Root MSE	DNAcontent__pg_ Mean
0.867869	1.966878	0.011616	0.590578

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	2	0.00344656	0.00172328	12.77	<.0001
Genomic_group	6	0.13221126	0.02203521	163.31	<.0001
accession(Genomic_g)	83	0.02209612	0.00026622	1.97	<.0001

Tests of Hypotheses Using the Type III MS for accession(Genomic_g) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genomic_group	6	0.13221126	0.02203521	82.77	<.0001

Table 6. 2: Duncan's Multiple Range Test 2C DNA value

Means with the same letter are not significantly different

Duncan Grouping	Mean	N	accession name
A	2.326	3	GT
A			
A	2.305	3	ITC0504FHIA01
B	1.829	3	ITC0048Valery
B			
B	1.827	3	ITC1122GrosMichel
B			
C B	1.823	3	ITC0084Mbwazirume
C B			
C B D	1.819	3	ITC0141Kagera
C B D			
C E B D	1.811	3	ITC0086Nyamwiihogora
C E B D			
C E B D	1.810	3	ITC0724Cocos
C E B D			
C E B D	1.808	3	ITC0083Igisahira
C E B D			
C E B D	1.806	3	Poyo
C E B D			
C E B D	1.805	3	ITC0154Mbirabire
C E B D			
C E B D	1.804	3	Kampala
C E B D			
C E B D	1.802	3	Pekera
C E B D			
C E B D	1.801	3	ITC0263Highgate
C E B D			
F C E B D	1.800	3	ITC0340PisangMasakHijau
F C E B D			
F C E B D G	1.797	3	ITC0168Imbogo
F C E B D G			
F C E B D G	1.795	3	ITC0170Bakururа
F C E B D G			
F C E B D G	1.795	3	ITC0163Inyova
F C E B D G			
F C E B H D G	1.777	3	Iholena upehupehu
F C E H D G			
F C E I H D G	1.769	3	Manzano BG Maui
F E I H D G			
F J E I H D G	1.764	3	Popoulu Hua Moa
F J E I H D G			
F J E I H D G	1.762	3	Kisukari
F J E I H G			
F J E I H K G	1.759	3	Maoli popoulu
F J E I H K G			
F J E L I H K G	1.756	3	KifutuKisii
F J L I H K G			
F J M L I H K G	1.745	3	ITC0583LFSJohnstone
F J M L I H K G			
F J M L I H K G	1.744	3	Embul
J M L I H K G			
J M L I H K G	1.742	3	Mboki
J M L I H K G			
J M L I H K G	1.742	3	Manzano Maui
J M L I H K G			
J M L I H K G	1.741	3	ITC0076Pome
J M L I H K			
N J M L I H K	1.733	3	ITC0207Prata
N J M L I H K			
N J M L I H K	1.732	3	Soth
N J M L I H K			
N J M L I H K	1.730	3	Raja puri Maui
N J M L I H K			
N J M L I H K	1.730	3	ExeraKisii
N J M L I H K			
N J M L I H K	1.727	3	Kakamega
N J M L I H K			
N J M L I H K	1.724	3	Iholena lele
N J M L I H K			
N J M L I H K O	1.723	3	ITC0769FiguePomeGeante
N J M L I H K O			
N J M L I H K O	1.722	3	ITC0127Kamaramasenge
N J M L I H K O			
N J M L I H K O	1.722	3	KamaramasengeK
N J M L I H K O			
N J M L I H K O	1.721	3	Manjano
N J M L I H K O			
N J M L I H K O	1.720	3	ITC0743FigueFamille
N J M L I H K O			
N J M L I H K O	1.720	3	ITC0649Foconah
N J M L I K O			
N J M L I K O	1.718	3	ITC0348Silk
N J M L I K O			
N J M L I K O	1.717	3	Manyatta
N J M L I K O			
N J M L I K O	1.716	3	Mysore
N J M L I K O			

AAAB tetraploids

AAA triploids

N	J	M	L	I	K	O	1.716	3	ITC0336ImprovedLFinger
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.715	3	SukariNdizi
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.714	3	ITC0535KluaiRoiWi
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.713	3	ITC0582LFNelson
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.712	3	Sweetyellow
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.712	3	ITC0962PrataAna
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.711	3	ITC0459NeyPoovan
N	J	M	L	I	K	O			
N	J	M	L	I	K	O	1.710	3	ITC1222SportofSilk
N	J	M	L		K	O			
N	J	M	L	P	K	O	1.707	3	UngoyesweetKisii
N	J	M	L	P	K	O			
N	J	M	L	P	K	O	1.705	3	ITC1441PisangCeylan
N		M	L	P	K	O			
N		M	L	P	K	O	1.702	3	ITC0987Auko
N		M	L	P	K	O			
N		M	L	P	K	O	1.701	3	Tall Brazilian Maui
N		M	L	P		O			
N		M	L	P		O	1.699	3	ITC0737Kingala1
N		M	L	P		O			
N		M	L	P		O	1.698	3	Peurto rican D plantain
N		M	L	P		O			
N		M	L	P		O	1.697	3	ITC0990Vunapope
N		M		P		O			
N		M		P		O	1.690	3	ITC1275Yangambi2
N				P		O			
N	Q			P		O	1.678	3	Popoulu ka'io
	Q			P		O			
	Q			P		O	1.665	3	ITC0087Kayinja
	Q			P					
	Q			P			1.652	3	Dippig Maui
	Q								
	Q						1.638	3	Bluggoe Maui
				R			1.299	3	Sucrier Maui
				R					
	S			R			1.290	3	HutiW
	S			R					
	S			R	T		1.278	3	Maia Hapai
	S			R	T				
	S			R	T		1.274	3	Injingu
	S			R	T				
	S	U		R	T		1.268	3	ITC1223Mshale
	S	U		R	T				
V	S	U		R	T		1.262	3	Mlelembo
V	S	U		R	T				
V	S	U		R	T		1.261	3	MuraruWB
V	S	U		R	T				
V	S	U		R	T		1.261	3	Kahuti
V	S	U		R	T				
V	S	U		R	T	W	1.251	3	ITC1253MjengaMicheldiploid
V	S	U		R	T	W			
V	S	U		R	T	W	1.246	3	HutiG
V	S	U		R	T	W			
V	S	U		R	T	W	1.245	3	ITC0428HigaBanksii
V	S	U			T	W			
V	S	U			T	W	1.243	3	ITC0480PisangBuntal
V	S	U			T	W			
V	S	U		X	T	W	1.238	3	ITC1358Ngu
V	S	U		X	T	W			
V	S	U		X	T	W	1.237	3	ITC0672PaRayong
V	S	U		X	T	W			
V	S	U		X	T	W	1.235	3	ITC0435PisangMasAyer
V		U		X	T	W			
V		U		X	T	W	1.232	3	ITC0392Datil
V		U		X	T	W			
V		U		X	T	W	1.231	3	MuraruMshareKisii
V		U		X	T	W			
V	Y	U		X	T	W	1.225	3	ITC0269NiyarmaYik
V	Y	U		X	T	W			
V	Y	U		X	T	W	1.222	3	ITC0714Kirun
V	Y	U		X		W			
V	Y	U		X		W	1.213	3	ITC1034Kunnan
V	Y			X		W			
V	Y			X		W	1.205	3	ITC0249Calcutta4
	Y			X		W			
	Y			X		W	1.202	3	MraruMlaluKisii
	Y			X					
	Y			X			1.183	3	NjuruKisii
	Y								
	Y			Z			1.172	3	ITC0245SafetVelchi
				Z					
				Z			1.127	3	ITC0246Cameroun
				A			1.072	3	ITC0094Balbisiana

ABB triploids

AA diploids

BB diploids

Table 6. 3: Duncan's Multiple Range Test for DNA_content_Mbp

Duncan Grouping										Mean Mbp	N	accession
					A					627	3	Sucrier Maui
					A							
		B			A					623	3	HutiW
		B			A							
		B			A		C			617	3	Maia Hapai
		B			A		C					
		B		D	A		C			615	3	Injingu
		B		D	A		C					
	E	B		D	A		C			612	3	ITC1223Mshale
	E	B		D	A		C					
	E	B		D	A		C	F		609	3	Mlelembo
	E	B		D	A		C	F				
	E	B		D	A		C	F		609	3	MuraruWB
	E	B		D	A		C	F				
	E	B		D	A	G	C	F		608	3	Kahuti
	E	B		D		G	C	F				
	E	B		D	H	G	C	F		604	3	ITC1253MjengaMicheldiploid
	E			D	H	G	C	F				
	E	I		D	H	G	C	F		601	3	HutiG
	E	I		D	H	G	C	F				
	E	I		D	J H	G	C	F		601	3	ITC0428HigaBanksii
	E	I		D	J H	G	C	F				
	E	I	K	D	J H	G	C	F		600	3	ITC0480PisangBuntal
	E	I	K	D	J H	G	C	F				
	E	I	K	D	J H	G	C	F		597	3	ITC1358Ngu
	E	I	K	D	J H	G	C	F				
	E	I	K	D	J H	G	C	F		597	3	ITC0672PaRayong
	E	I	K	D	J H	G	C	F				
	E	I	K	D	J H	G	C	F		596	3	ITC0435PisangMasAyer
	E	I	K	D	J H	G		F				
	E	I	K	D	J H	G		F		595	3	ITC0392Datil
	E	I	K	D	J H	G		F				
	E	I	K	D	J H	G	L	F		594	3	MuraruMshareKisii
	E	I	K		J H	G	L	F				
	E	I	K	M	J H	G	L	F		591	3	ITC0269NiyarmaYik
		I	K	M	J H	G	L	F				
	N	I	K	M	J H	G	L	F		590	3	ITC0714Kirun
	N	I	K	M	J H	G	L	F				
	N O	I	K	M	J H	G	L	F		588	3	ITC0048Valery
	N O	I	K	M	J H	G	L	F				
	N O	I	K	M	J H	G	L	F		588	3	ITC1122GrosMichel
	N O	I	K	M	J H	G	L					
	N O	I	K	M	J H	G	L	P		586	3	ITC0084Mbwazirume
	N O	I	K	M	J H		L	P				
	N O	I	K	M	J H		L	P		585	3	ITC1034Kunnan*
	N O	I	K	M	J H		L	P				
	N O	I	K	M	J H		L	P		585	3	ITC0141Kagera
	N O	I	K	M	J H		L	P				
	N O	I	K	M	J H	Q	L	P		583	3	ITC0086Nyamwiihogora
	N O	I	K	M	J H	Q	L	P				
	N O	I	K	M	J H	Q	L	P		582	3	ITC0724Cocos
	N O	I	K	M	J H	Q	L	P				
	N O	I	K	M	J H	Q	L	P		582	3	ITC0083Igisahira
	N O	I	K	M	J H	Q	L	P				
	N O	I	K	M	J H	Q	L	P		581	3	ITC0249Calcutta4
	N O	I	K	M	J	Q	L	P				
	N O	I	K	M	J	Q	L	P		581	3	Poyo
	N O	I	K	M	J	Q	L	P				
	N O	I	K	M	J	Q	L	P		581	3	ITC0154Mbirabire
	N O	I	K	M	J	Q	L	P				
	N O	I	K	M	J	Q	L	P		580	3	Kampala
	N O	I	K	M	J	Q	L	P				
	N O	I	K	M	J R	Q	L	P		580	3	MraruMlaluKisii
	N O	I	K	M	J R	Q	L	P				
	N O	I	K	M	J R	Q	L	P		580	3	Pekera
	N O	I	K	M	J R	Q	L	P				
	N O	I	K	M	J R	Q	L	P		579	3	ITC0263Highgate
	N O	I	K	M	J R	Q	L	P				
S	N O	I	K	M	J R	Q	L	P		579	3	ITC0340PisangMasakHijau
S	N O		K	M	J R	Q	L	P				
S	N O	T	K	M	J R	Q	L	P		578	3	ITC0168Imbogo
S	N O	T	K	M	R	Q	L	P				
S	N O	T	K	M	R	Q	L	P		577	3	ITC0170Bakururа
S	N O	T	K	M	R	Q	L	P				
S	N O	T	K	M	R	Q	L	P		577	3	ITC0163Inyoya
S	N O	T		M	R	Q	L	P				
S	N O	T		M	U R	Q	L	P		572	3	Iholena upehupehu*
S	N O	T		M	U R	Q		P				
S	N O	T		M	U R	Q	V	P		571	3	NjuruKisii
S	N O	T		M	U R	Q	V	P				
S	N O	T	W	M	U R	Q	V	P		569	3	Manzano BG Maui
S	N O	T	W		U R	Q	V	P				
S	N O	T	W		U R	Q	V	P		567	3	Popoulu Hua Moa
S	O	T	W		U R	Q	V	P				
S	O	T	W	X	U R	Q	V	P		567	3	Kisukari
S	O	T	W	X	U R	Q	V	P				
S	O	T	W	X	U R	Q	V	P		566	3	Maoli popoulu
S	O	T	W	X	U R	Q	V	P				
S	O	T	W	X	U R	Q	V	P		566	3	ITC0245SafetVelchi*

Composed of A only except *

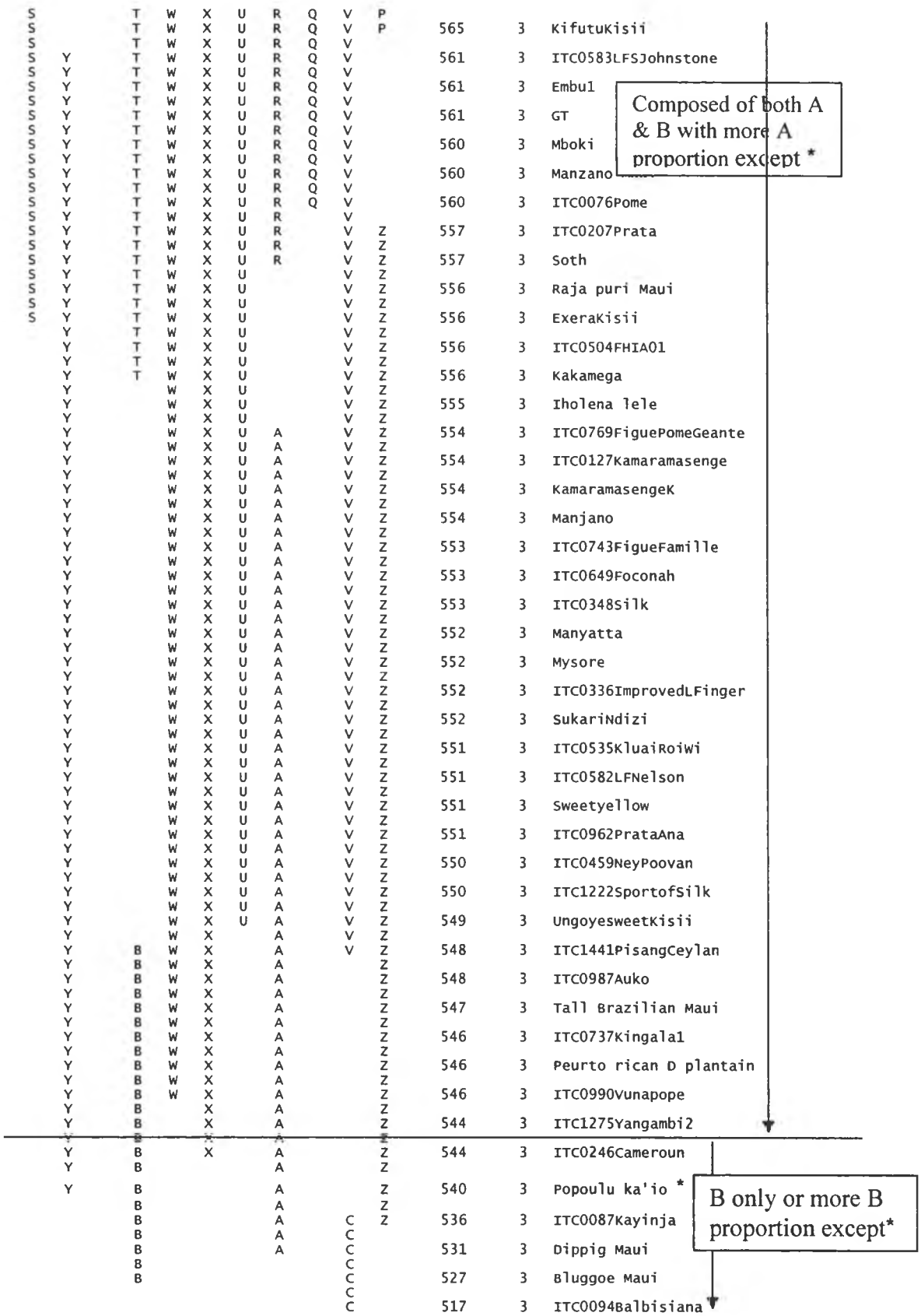

Composed of both A & B with more A proportion except *
B only or more B proportion except*

Table 6. 4: Mean 2C DNA and genome size of the various banana genomic groups

Genomic group	2C DNA	Monoploid size (pg)	Monoploid size (Mbp)
AAAB	2.315[a]	0.579[c]	559[c]
AAA	1.808[b]	0.603[b]	582[b]
AAB	1.724[c]	0.575[c]	555[c]
ABB	1.652[d]	0.551[d]	531[d]
AA	1.245[e]	0.622[a]	601[a]
AB	1.193[f]	0.596[b]	575[b]
BB	1.099[g]	0.549[d]	530[d]
Critical value	0.032	0.013	13
R^2	0.985	0.746	0.746
CV	2.02	2.251	2.251

Table 6. 5: Duncan's Multiple Range Test 2C DNA value of accessions analyzed in ILRI Nairobi

Means with the same letter are not significantly different

Duncan Grouping	2C DNA Mean	N	accession
A	2.260	3	GT Kisii
B	1.855	3	Paji Zanzibar
C	1.801	3	Mutsari Rwanda
C	1.780	3	Kioberabaswa Rwanda
C E	1.775	3	Sukari Ndiizi Entebbe
F E	1.754	3	Pukusa Zanzibar
F E	1.751	3	Prata Rwanda
F E	1.751	3	Kikonde Zanzibar
F E	1.736	3	Kamaramasenge Rwanda
F	1.723	3	Gisubi Rwanda
F	1.722	3	Kijakazi Zanzibar
F	1.722	3	Sukari Ndizi Kisii
G	1.251	3	Kipaka Zanzibar
G	1.223	3	Kisukari Zanzibar
G	1.222	3	Kisubi Entebbe
H	1.162	3	Gisubi Kagongo Rwanda

AAAB tetraploids

AAA triploids

AA diploids

6.4. Discussion

The data show that the flow cytometry method has a high accuracy when used in ploidy determination. Tetraploids, triploids, and diploids were clearly separated by this technique.

Using flow cytometry may be an alternative method for determining the genomic composition of bananas, provided that enough standard banana clones of known composition are available for comparison. The use of internal standard, replications, and good experimental techniques are essential.

Because of the variation within both the *acuminata* and the *balbisiana* species, there were also variations within the various genome groups. Batos *et al.* (2005) showed that, within *M. acuminata*, even though the differences between the accessions were small (3.4%) they were significant. Lysak *et al.* (1999) and Dolezel *et al.* (1994) found that the A and B genomes of *Musa* differ in size. Lysak *et al.* (1999) found that the B genome was generally smaller than the A genome, by 12% on average (small but significant); no variation was found in genome size amongst the *M. balbisiana* in the same study. In this study, there were significant differences in the 2C DNA value within the AAB group of bananas, possibly because of the variation in the 2C DNA value between accessions with an AA genomic composition. Lysak *et al.* (1999) observed statistically significant differences in the 2C DNA within the AA accessions (1.225 to1.274 pg). The results reported here were in agreement with those obtained by these workers.

A number of consistencies exist between these results and those obtained by previous workers. In their study, Lysak *et al.* (1999) used ITC 0246 Cameroun BB

and BB ITC0094, like those used in this study. Although the two BB sub-species were not significantly different in 2C DNA values in both studies, ITC0246 Cameroun had a higher 2C DNA in both studies.

This study demonstrated that the monoploid size of the A, or predominantly A, genome composition banana accessions are bigger than those of the B, or predominantly B genome banana accessions. There was a general trend to smaller monoploid genome sizes in polyploids, compared to diploids. Diploids need intact genomes to be able to function and live, unlike polyploids that can lose portions of their chromosomes without it being lethal.

Some interesting results were found for some accessions. Accession GT was verified as a tetraploid AAAB, while ITC0987 Auko, ITC0990 Vunapope, ITC0459 Ney Poovan, and ITC0127 Kamramasenge were verified to be triploid AAB, instead of diploid AB, as previously thought. Accession ITC0392 Datil was verified to be AA instead of an AB. It was also interesting to note that Iholena upehupehu had a relatively big monoploid size and 2C DNA value. Simmonds and Stover (1987) had earlier classified it as belonging to the triploid AAA group, using the 15 character score method (Simmonds and Shepherd 1955); however, the molecular data in this study shows it clustering with the AAB Polynesian and African plantains.

CHAPTER 7

THE HORTICULTURAL TRAITS STUDIES OF THE EAST AFRICAN *MUSA* AAB "APPLE" AND AA "MURARU" DESSERT BANANAS

7.0. Introduction to horticultural banana evaluation

Banana is a food crop, food security crop in times of food shortage, horticultural and a cash crop. The crop management by banana growers depends upon the reason why they are growing the crop. As a horticultural fruit crop, however, the dessert bananas are the most important. In the world, the prevalent commercial dessert bananas for export are the triploid AAA Cavendish clones. Other dessert bananas are grown for local consumption in the growing regions. In East Africa most of the dessert bananas grown are consumed locally and the export market is of minor importance.

Being an horticultural fruit crop, it was important to evaluate the various AAB "Apple" and AA "Muraru" dessert banana accessions for valuable horticultural traits. Banana farmers need access to appropriate technologies, including the best cultivars for intensive production. Banana is one of the very few crops in which cultivars developed by controlled breeding have not replaced those derived from natural evolution (Rowe and Rosales 1996). Regardless of the source, cultivar recommendations for small growers must be made from an accurate knowledge of national or regional resources. Evaluation under local conditions provides more accurate information regarding cultivar characteristics. To be able to increase

productivity of AAB "Apple" and AA "Muraru" dessert bananas and to make cultivar recommendation to East African growers, it was important to evaluate the horticultural potential of the clones in the field. In this study, that involved two experiments, one for the AAB "Apple" bananas and a second for the AA "Muraru" banana. The accessions were evaluated for several horticultural traits deemed to be important.

7.1. Specific objectives

The study of horticultural traits evaluation had the following objectives:

1. To determine the horticultural value of "Sukari Ndizi" and "Muraru" bananas relative to other AAB "Apple" dessert bananas; and
2. To identify the AAB "Apple"and AA "Muraru"cultivars that are superior in horticultural traits for recommendation to East African farmers.

7.2. Materials and methods

7.2.1. Study site biophysical characteristics

The trial site (Figure 7.1) was at KARI-Kisii, Kenya, at latitude 00^0 41'S and longitude 34^0 47'E in the Upper Midland one (UM_1) agroecological zone (Jaetzold and Schmidt 1982). KARI-Kisii is situated on a rolling upland with an average slope of 8%. The site altitude was 1752 metres above mean sea level and has an average annual rainfall of 1700mm. Figures 7.2 and 7.3 show the weather data during the study period.

The soil is an oxil tropudalf, according to the USDA soil taxonomy (1975), or Eutric Nitosol (acid soils with a very thick layer of clay accumulation) by FAO/UNESCO (1974, 1987) soil classification. The dark reddish-brown 120-160cm deep, well drained soil has a clay texture and is commonly referred to as deep brown loamy clay. The soil reaction is slightly to moderately acidic (pH= 5.2-5.6). The soil has adequate amounts of exchangeable calcium, magnesium, potassium and sodium bases, but only marginal amounts of organic carbon (organic matter) and total nitrogen. It is deficient in extractable phosphorous (Siderius and Muchena 1977).

7.2.2. Treatments and trial design

A total of 25 banana accessions were evaluated. The 15 "Apple" dessert bananas and ten "Muraru" dessert banana accessions (Tables 7.1a and 7.2a) used in the trial are a subset of the accessions used for morphological and molecular studies (Table 4.1). These cultivars were obtained from the KARI-Kisii and KARI-Thika banana collections. Banana cultivars from the other East African countries were not included in this trial, because it was not possible to get the material to the trial site at the time.

Two experiments were established; one for the AAB "Apple" banana accessions and another for the AA "Muraru" banana accessions. The trials were laid out in a Randomized Complete Block Design (RCBD) with three replications (Tables 7.1b and 7.2b). The experimental design was based on single factor banana accessions; treatments were randomly assigned to the plots within each block, using a random number table. The experimental units or plots measured 6m x 4m ($24m^2$)

with 4 plants spaced at 3m x 2m giving a density of one plant per $6m^2$. Data were collected on all four plants in each plot. The replicates, or blocks, run parallel to the contours and perpendicular to the slope, such that the soil fertility within each block was more or less homogenous. The AAB "Apple" experiment was surrounded by guard rows consisting of Apple banana plants cultivar Exera and the Muraru experiment was surrounded by guard rows consisting of Muraru Mshale.

Table 7. 1a: The AAB "Apple" banana accessions used in the study and their plot positions

"Apple" accession (Treatment)	**Replication 1 (plot number)**	**Replication 2 (plot number)**	**Replication 3 (plot number)**
GT	1	23	37
Sukari Ndizi	2	27	40
Embu	3	26	32
Kakamega	4	22	41
Kamaramasenge	5	17	35
Ungoye sweet	6	20	44
Manjano	7	29	43
Mboki Msukari	8	24	38
Mysore	9	19	31
Kifutu	10	25	42
Soth	11	18	34
Exera	12	28	39
Manyatta	13	16	36
Wangae (Kisii)	14	30	33
Wangae (Thika)	15	21	45

Table 7.1b: Field layout for the AAB "Apple" banana trial

	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
BK1	*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	*
BK2	*	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16	*
BK3	*	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	*
	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Key: * 2 rows of banana plants acting as guard rows; BK: Block or replicate

Table 7. 2a: The AA "Muraru" banana accessions used in the study and their plot positions

Muraru accession (Treatment)	**Replication 1 (plot number)**	**Replication 2 (plot number)**	**Replication 3 (plot number)**
TT2	1	16	24
Muraru	2	19	26
Mraru Mlalu	3	14	22
Muraru Mshare	4	15	27
Njuru	5	11	23
Muraru white	6	13	29
Muraru red	7	20	28
Kamunyilya	8	17	25
Maji maji	9	12	30
Makhughu	10	18	21

Table 7.2b: Field layout for the AA "Muraru" banana trial

	*	*	*	*	*	*	*	*	*	*	*	*
BK1	*	1	2	3	4	5	6	7	8	9	10	*
BK2	*	20	19	18	17	16	15	14	13	12	11	*
BK3	*	21	22	23	24	25	26	27	28	29	30	*
	*	*	*	*	*	*	*	*	*	*	*	*

Key: * 2 rows of banana plants acting as guard rows; BK: Block or replicate

Figure 7. 1: The AAB "Apple" and AA "Muraru" banana accessions trial site at Kenya Agricultural Research Institute (KARI), Kisii, Kenya.

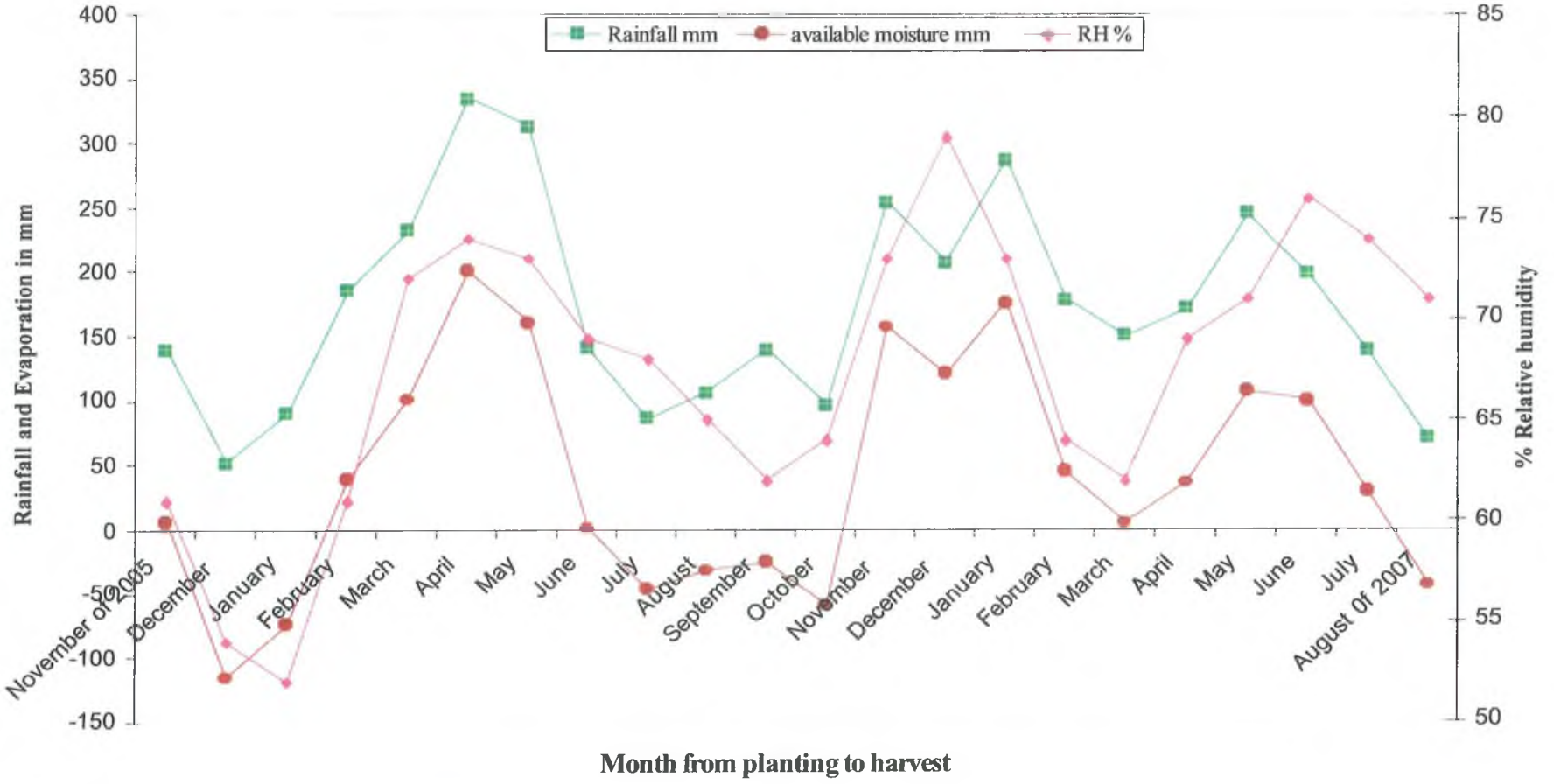


Figure 7. 2: Rainfall moisture and Relative humidity trend at Kenya Agricultural Research Institute (KARI), Kisii, Kenya, during the trial period, November 2005 - August 2007.

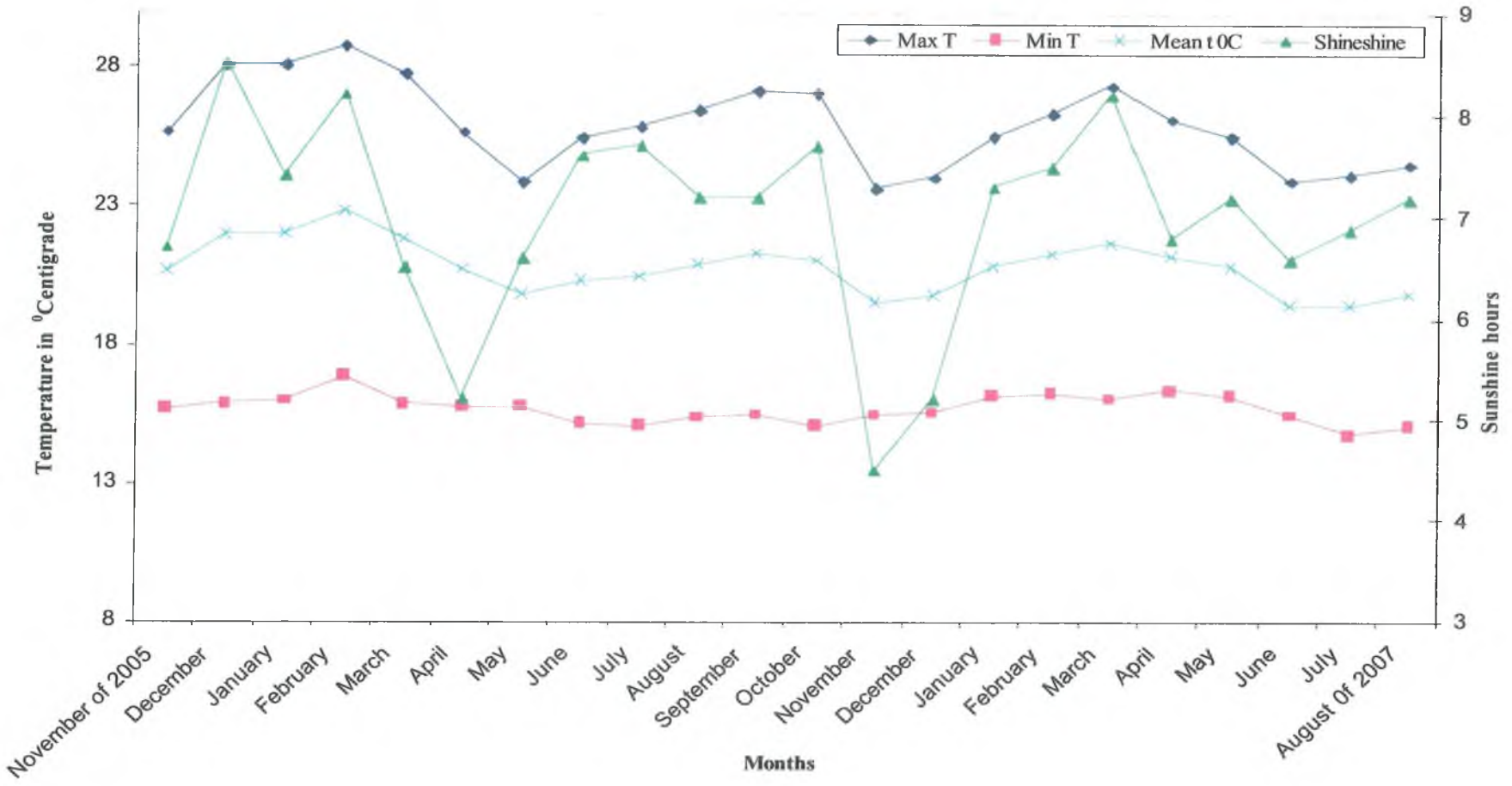


Figure 7. 3: Sunshine, minimum, maximum and mean temperatures at Kenya Agricultural Research Institute (KARI), Kisii, Kenya, during the study period, November 2005 - August 2007.

7.2.3. Crop management

7.2.3.1. Planting

The banana plants were planted in October 27th in 60cm x 60cm x 60cm holes. Topsoil from the upper 30cm layer was separated from the bottom soil and mixed with well-rotted farmyard manure at the recommended rate of 7.5 tons per hectare (30 kilogram per hole). Diammonium phosphate (DAP) inorganic fertilizer was added at the rate of 200grams per hole, mixed, and then the topsoil was returned to the hole for planting the young sword suckers. The subsoil from the lower 30cm of the profile was used to back-fill the hole.

7.2.3.2. Field maintenance

The banana trials were rain-fed. After six months, in April 2006, the plants were given a topdress fertilization with calcium ammonium nitrate (CAN) at 100 grams per plant, which coincided with the April rains. In November 2006, plants were topdressed with 100 grams per mat CAN. The experimental fields were kept weed-free with the herbicide (Roundup) sprayed between the rows; the mats were weeded manually. This method ensured that the herbicides did not get into the root and plant to interfere with growth. Plant health was monitored and records of disease incidences and severity were kept.

7.2.4. Data collection

7.2.4.1. Choice of parameters and data collection

The parameters used in data collection were chosen based on the literature and knowledge of the crop. The following 20 parameters in Table 7.3 were used.

Table 7. 3: Horticultural traits evaluated for data collection for both AAB "Apple" and AA "Muraru" trials

Traits used in horticultural evaluation
Monthly leaf emergence
Monthly sucker emergence
Total number of leaves produced by flowering
Leaves remaining at flowering
Leaves remaining at harvest
Total number of suckers at harvest
Plant girth at flowering
Plant height at flowering
Days taken from planting to flowering
Days taken from planting to harvest
Days for fruit filling
Bunch weight
Leaf area
Number of hands per bunch
Number of fruits per hand
Number of fruits per bunch
Hand weight
Fruit weight
Fruit length
Fruit circumference
Yield in tons per hectare per year

7.2.4.1.1. Monthly sucker and leaf emergence and other leaf characters

Data were collected on leaf emergence starting in January 2006, 2 months after planting, and data collection on sucker emergence commenced in August 2006, eight months after planting. Between January and August 2006, no suckers were produced. Data on the number of suckers and leaves that had emerged during the previous month were collected on the 1st day of the month. The new leaves and suckers were marked with a permanent paint to ensure no double counting. Data taken on leaf emergence stopped when flowering occurred. Sucker emergence was recorded until harvest to get the total number of suckers produced. The total and average monthly leaf and sucker production were calculated. The number of green leaves present at flowering was recorded, since some leaves died as other were produced during the life of the plant. Leaves retained until harvest were also recorded at harvest time. Other data recorded on the harvest date on 5 randomly selected leaves were the petiole length, the length (L) of the lamina of leaf and width (W) of leaf. The leaf area (LA) was then calculated from the length and width of the leaf (LA = 0.83LW (Robinson *et al.* 1993) and the average recorded.

7.2.4.1.2. Days to floweing, plant height, plant girth

Data on plant height, plant girth, and days to flowering were taken at flowering. Flowering in banana occurs after the flag leaf has been produced; the banana plant exerts the inflorescence which bends over within a week. This was recorded as the date of flowering, and the calculation of "days to flowering" is made by determining the number of days since planting. On the same day, plant height (the

measurement of the pseudostem height from ground level to where the inflorescence appeared) and plant girth, which is the circumference of the pseudostem at 1m above ground level, were recorded.

7.2.4.1.3. Days to harvest, bunch weight, other bunch characteristics

Although many authors record the date of harvest as that date when a fruit cross- section reaches "¾ -round," in this study, harvest time for the AAB "Apple" clones was the date the first fruit turned yellow and for the AA "Muraru," when the first fruit turned green-yellow. This allowed for more objectivity in determining the date the dessert banana bunch is ready for harvest. The determination of the ¾ -round stage of maturity was regarded as being too subjective. The days from flowering to harvest and days from planting to harvest were recorded. The harvested bunch weight and the number of hands per bunch were recorded. Data pertaining to individual hand and fruit measurements, including number of fingers per hand, finger length and circumference, and weight of hand and fruit were all recorded on the third hand of the bunch from the top for all accessions.

7.2.4.1.4. Yield potential

The yield potential (YLD, t ha^{-1} $year^{-1}$) was defined as: YLD = BW X 365 X 1667 / (DH X 1000), where BW and DH are bunch weight in kilograms per accession and days to harvest, respectively. Planting density was 1667 ha^{-1}, and 365 was the constant value for days in one year.

7.2.5. Data analysis

7.2.5.1. Analysis of variance (ANOVA) and treatment comparisons

Data were analyzed by Analysis of Variance (ANOVA), using the General Linear Model procedure of SAS software (SAS Institute, 1994). Each variable was analyzed across all of the banana accessions in each study. Tables 7.3 and 7.4 show the format for the ANOVA and the expectations of mean squares. Variables found to have significant differences between the cultivars were further subjected to Duncan's multiple range tests for means separation to see which cultivars were significantly different. For the AAB "Apple" banana trial, the same data were further subjected to ANOVA based on the various grouping (Prata, Silk, Mysore, and "Sukari Ndizi"). To detect correlated responses among the variables, correlation analysis was done.

Table 7. 4: Analysis of variance table for the AAB "Apple" banana trial

Source of variation for AAB "Apple"	df	Composition of ms	ms	Appropriate F -test
Blocks	2	$\sigma_s^2+s\sigma_e^2+ ag\sigma_r^2$	m_1	m_1/m_4
Among groups	4	$\sigma_s^2+s\sigma_e^2+ r\sigma_{a(g)}^2 + ra\sigma_g^2$	m_2	m_2/m_3
Within group	10	$\sigma_s^2+s\sigma_e^2+ r\sigma_{a(g)}^2$	m_3	m_3/ m_4
Within accession within blocks (error)	28	$\sigma_s^2+s\sigma_e^2$	m_4	m_4/m_5
Sample	135	σ_s^2	m_5	-
Total	179	-	-	-

g = number of groups, r = number of replicates, a = accession within group, s = sample

The mathematical model used for the analysis of variance is as follows:

$Y_{ij} = \bar{Y}.. + T_i + B_j + e_{ij}$

Where:

Y_{ij} = an observation

$\bar{Y}..$= the experimental mean

T_i = the treatment effect

e_{ij} = the residual or experimental error

B_j = the block effect

$_i$= a particular treatment

$_j$ = a particular block

Table 7. 5: Analysis of variance table for the AA "Muraru" banana trial

Source of variation for AA "Muraru"	df	Composition of ms	ms	Appropriate F -test
Blocks	2	$\sigma_s^2+s\sigma_e^2+ t\sigma_r^2$	m_1	m_1/m_3
Treatments	9	$\sigma_s^2+s\sigma_e^2+ r\sigma_t^2$	m_2	m_2/m_3
Error	18	$\sigma_s^2+s\sigma_e^2$	m_3	m_3/m_4
Sample	90	σ_s^2	m_5	-
Total	119	-	-	-

r = number of replicates, t = number of treatments, s = sample

The mathematical model used for the analysis of variance is as follows:

$Y_{ij} = \bar{Y}.. + T_i + B_j + e_{ij}$

Where:

Y_{ij} = an observation

$\bar{Y}..$= the experimental mean

T_i = the treatment effect

e_{ij} = the residual or experimental error

B_j = the block effect

$_i$= a particular treatment

$_j$ = a particular block

7.2.5.2. Regression

Regression and correlation analysis was done for bunch weight, days to harvest, and yield potential. Yield was the dependent variable, and bunch weight and days to harvest were explanatory variables in the analysis.

7.3. Results for AAB "Apple" banana trial

Significant differences among AAB "Apple' accessions were obtained in all traits evaluated, (Tables 7.6 and 7.7). Similarly significant differences occurred in all traits evaluated among the various AAB "Apple banana groupings, namely, Prata, Silk, "Sukari Ndizi", Mysore), and the GT tetraploid (Table 7.8).

7.3.1. Days to flowering of AAB "Apple" bananas

The mean number of days to flowering was 422, and ranged from 362 to 508 days (Tables 7.6 and 7.8). Mysore took significantly longer to flower, compared to all other AAB "Apple" banana groupings (Figures 7.4). The tetraploid GT, the Prata (Manyatta, Soth, Exera, and Kifutu), and the Silk accessions (Mboki Msukari, Manjano and Ungoye sweet) also took significantly longer to flower compared to the

"Sukari Ndizi" accessions (Wangae Kisii, Wangae Thika, Embu, Kamaramasenge, Kakamega and Sukari Ndizi) (Table 7.6).

7.3.2. Period between flowering and harvest of AAB "Apple" bananas

The mean number of days between flowering and harvest over all accessions was 171 (Table 7.6); the range was from 130 to 211days. The Prata accessions took significantly longer from flowering to harvest than the other AAB "Apple" groupings (Figure 7.4), and "Sukari Ndizi" accessions took significantly longer from flower to harvest than the Silk accessions and the tetraploid GT. The Silk accessions and the tetraploid GT were significantly different from each other in this characteristic.

Table 7. 1: Analysis of vegetative trait means, days from planting to harvest, days from flower to harvest of *Musa* AAB "Apple" banana accessions grown in Kisii, Kenya

Accession	Days to Flowering	Days to Harvest	Flowering to Harvest (days)	Plant Height (meters)	Plant Girth (centimeters)	Total Leaves	Leaves at Flowering	Leaves at Harvest	Leaf Area (cm^2)	Total Suckers
GT	451bc	603b	152gh	2.79b	54.9a	37bc	13bc	9c	10509bc	11e
Sukari Ndizi	362h	547c	186d	2.44cd	45.8de	32f	12bcd	6e	9759cdef	17ab
Embu	391fg	547c	156efg	2.44cd	41.9fg	32ef	12bcd	7de	9332efg	15bc
Kakamega	364h	542c	178cde	2.48cd	45.3de	32ef	11de	7de	9732bcd	17ab
Kamaramasenge	390fg	547c	158cde	2.41cd	40.7g	31f	11de	6e	8244h	12de
Wangae Thika	402ef	568bc	167defg	2.47cd	44.5de	34de	11de	8d	8807fgh	18ab
Wangae Kisii	372fg	544c	173cdef	2.36d	43.8def	34def	12bcd	7de	9614def	19a
Ungoye sweet	422de	564c	142gh	2.55c	43.4ef	36cd	14d	5f	9016fgh	12de
Manjano	432cd	563c	130h	2.55c	46.1d	39ab	13bc	4f	7258i	13cde
Mboki Msukari	434cd	603b	169cdef	2.48cd	46.2d	38bc	14b	6e	8404h	15bc
Manyatta	466b	653a	187bcd	3.33a	52.8a	41a	16a	11ab	10699ab	16bc
Kifutu	445bc	656a	211a	3.23a	53.2a	38bc	16a	11ab	11290a	15bc
Soth	442bcd	651a	209ab	3.20a	53.4a	39ab	16a	12a	10262bcd	13cde
Exera	453bc	645a	194abc	3.23a	52.0b	40ab	16a	10b	9864cde	17ab
Mysore	511a	670a	162efg	3.26a	48.5c	40ab	14b	9c	9750bcd	12de
Mean	422	594	171	2.7	47.5	36	13	8	9503	15
CV	7.2%	4.5%	15.9%	5.7%	5.8%	7.8%	11.9%	16.9%	9.5%	24.6%

Means in the same column with the same letter/s coding are not significantly different, according to DMRT at $p \leq 0.05$.

Table 7. 2: Analysis of potential yield, bunch weight and fruit trait means of *Musa* AAB "Apple" banana accessions grown in Kisii, Kenya

Accession	Potential yield (ton ha^{-1} yr^{-1})	Bunch Weight (Kg)	Hands per Bunch	Fruit per Hand	Hand Weight (grams)	Fruit Weight (grams)	Fruit in Bunch	Length of Finger (cm)	Circumference of Finger (cm)
GT	14.3c	14.1c	8.3b	13.0b	1485c	118c	108b	16.1bc	14.5a
Sukari Ndizi	8.7ef	7.9de	7.8bcd	12.5b	989defg	79ef	97bc	13.7ef	12.1bc
Embu	8.7ef	7.8de	8.0c	12.5b	972efg	78ef	101bc	13.6ef	11.8bcd
Kakamega	8.6ef	7.7de	8.1b	12.8b	982defg	75ef	104bc	13.4ef	12.0bc
Kamaramasenge	7.5fg	6.8e	7.1def	11.0c	833g	75ef	78d	13.1f	11.3cd
Wangae Thika	9.7e	9.0d	8.2b	12.4b	1131de	92d	101bc	14.7de	12.3b
Wangae Kisii	8.8ef	7.8de	8.2b	12.7b	936fg	74f	104bc	14.1ef	12.2bc
Ungoye sweet	8.8ef	8.1d	6.5f	10.8c	1168d	107c	70d	17.3b	11.8bcd
Manjano	7.2g	6.6e	6.5f	10.8c	912fg	85def	70d	16.8bc	11.8bcd
Mboki Msukari	8.6ef	8.4d	6.9f	10.7c	1051def	88de	74d	16.9b	12.6b
Manyatta	15.3bc	16.4b	7.0ef	13.3b	1958b	149b	92c	21.8a	14.5a
Kifutu	15.8ab	16.9ab	7.2def	13.1b	2260a	174a	94c	21.8a	14.8a
Soth	16.9a	18.0a	7.1def	13.2b	2296a	175a	93c	21.3a	15.0a
Exera	15.0bc	15.9b	7.3cde	12.8b	2094b	164a	94c	20.8a	14.6a
Mysore	12.8d	14.0c	12.8a	17.3a	1068def	62g	224a	15.5cd	10.9d
Mean	11.1	11.0	8	13	1342	106	100	16.7	12.8
CV	13.3%	13.6%	10.1%	8.4%	15.3%	13.9%	13.5%	9%	8.2%

Means in the same column with the same letter/s coding are not significantly different, according to DMRT at $p \leq 0.05$.

Table 7. 3: Analysis of important horticultural trait means among *Musa* AAB "Apple" taxa in Kisii, Kenya

Grouping	Days to Flowering	Days to Harvest	Flower to Harvest	Plant Height	Plant Girth	Total Leaves	Leaves at Flowering	Leaves at Harvest	Leaf Area (cm^2)	Total Suckers
"Sukari Ndizi"	380d	550e	169b	2.43c	43.7d	33c	12c	7c	9248b	16a
Prata	451b	652b	200a	3.24a	52.9b	39a	16a	11a	10529a	15ab
Silk	429c	577d	147c	2.52c	45.22d	38b	13b	5d	8226c	13bc
Mysore	511a	670a	162bc	3.26a	48.53c	40a	14b	9b	9750b	10cd
GT tetraploid	451b	603c	152c	2.83b	54.9a	37b	14b	9b	10509a	9d
Mean	422	594	171	2.7	47.5	36	13	8	9503	15
CV	7.2%	4.5%	15.9%	5.7%	5.8%	7.8%	11.9%	16.9%	9.5%	24.6%

Grouping	Yield (ton ha^{-1} yr^{-1})	Bunch Weight (Kg)	Hands per Bunch	Fruit per Hand	Fruit per Bunch	Hand Weight (Kg)	Fruit Weight (grams)	Fruit Length (cm)	Fruit Circumference (cm)
"Sukari Ndizi"	8.6d	7.8c	8b	12c	97c	0.974c	79d	13.8d	11.9b
Prata	15.7a	16.8a	7c	13b	93c	2.152a	165a	21.5a	14.7a
Silk	8.2d	7.7c	7c	11d	72d	1.043c	93c	16.5b	12.2b
Mysore	12.8c	14.0b	13a	18a	225a	1.068c	62e	15.5c	10.8c
GT tetraploid	14.3b	14.1b	8b	13b	109b	1.485b	118b	15.9bc	14.4a
Mean	11.1	11.0	8	13	100	1342	106	16.7	12.8
CV	13.3%	13.6%	10.1%	8.4%	13.5%	15.3%	13.9%	9%	8.3%

Means in the same column with the same letter/s coding are not significantly different, according to DMRT at $p \leq 0.05$.

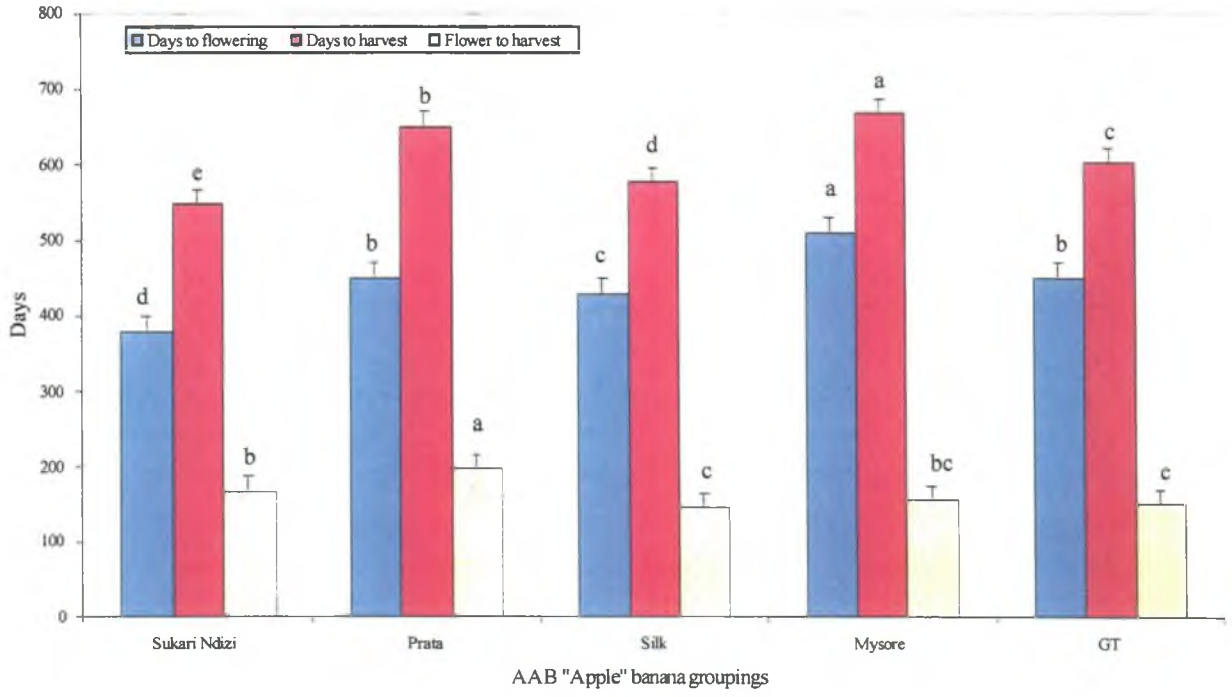


Figure 7. 4: Days taken to flowering, days to harvest, and fruit filling period for AAB "Apple" banana groupings.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

7.3.3. Days to harvest of AAB "Apple" bananas

Days from planting to harvest was the sum of the days from planting to flowering and days from flowering to harvest. The mean number of days to harvest for AAB "Apple" bananas was 594, and the range was from 542 to 670 days. The Mysore took significantly longer to reach maturity than all other groupings followed by the Prata, the tetraploid GT, the Silk and the "Sukari Ndizi" groupings in that order (Tables 7.6 and 7.8, and Figure 7.4). The "Sukari Ndizi" accessions required the least time to produce harvestable bunches, as a result of fewer days taken to flowering.

7.3.4. Plant height at flowering of AAB "Apple" bananas

The mean plant height for the AAB "Apple" banana accessions was 2.7 meters. The range of plant height was from 2.37 to 3.33 meters. The Prata and Mysore groupings were significantly taller (Tables 7.6 and 7.8) than the other AAB "Apple" accessions. The tetraploid GT was also significantly taller than the "Sukari Ndizi" and Silk accessions.

7.3.5. Plant girth at flowering of AAB "Apple" bananas

The mean plant girth of "Apple" banana accessions was 47.5 centimeters; the range of plant girth was between 40.7 and 54.9 centimeters. The tetraploid GT had significantly larger girth than all the AAB "Apple" groupings (Table 7.8). The GT was however not significantly different from Soth, Manyatta and Exera accessions of the Prata grouping (Table 7.6). The Prata accessions had significantly larger girths, compared to other AAB "Apple" bananas accessions. The Mysore had significantly bigger girth than the Silk and "Sukari Ndizi" groupings (Table 7.8) but the later two groupings were not significantly different in girth. The Kamaramasenge accession of the "Sukari Ndizi" grouping (Table 7.6) had significantly smaller girth than all AAB "Apple" accessions except Embu accession of "Sukari Ndizi" grouping.

7.3.6. Total number of leaves of AAB "Apple" bananas

The mean total number of leaves produced by the AAB "Apple" banana accessions was 36; the range was between 31 and 41 leaves. There were no significant differences in the total number of leaves produced between the Prata and

Mysore groupings, but these produced significantly more leaves than the tetraploid GT and the Silk grouping that were themselves not different, but had significantly more leaves than the "Sukari Ndizi" grouping (Table 7.8 and Figure 7.5).

7.3.7. Leaves at flowering of AAB "Apple" bananas

The mean number of leaves on banana plants at flowering was 13, with a range of 11 to 16 leaves. The Prata grouping had significantly more leaves at flowering than the Mysore, Silk, "Sukari Ndizi," and GT accessions (Table 7.8). The total number of leaves of Mysore was, however, not significantly different from the Exera and Soth, of the Prata grouping. "Sukari Ndizi" accessions had significantly fewer leaves present at flowering, compared to other groupings (Figure 7.5).

7.3.8. Leaves at harvest of AAB "Apple" bananas

The mean number of leaves at harvest was 8, and the range was from 4 to 12 leaves. The Prata groupings had significantly more leaves at harvest than all the other groupings (Table 7.8 and Figure 7.5). The tetraploid GT and the Mysore had more leaves at harvest than "Sukari Ndizi", but were not significantly different from each other. The Silk accessions had significantly fewer leaves at harvest than all the other groupings.

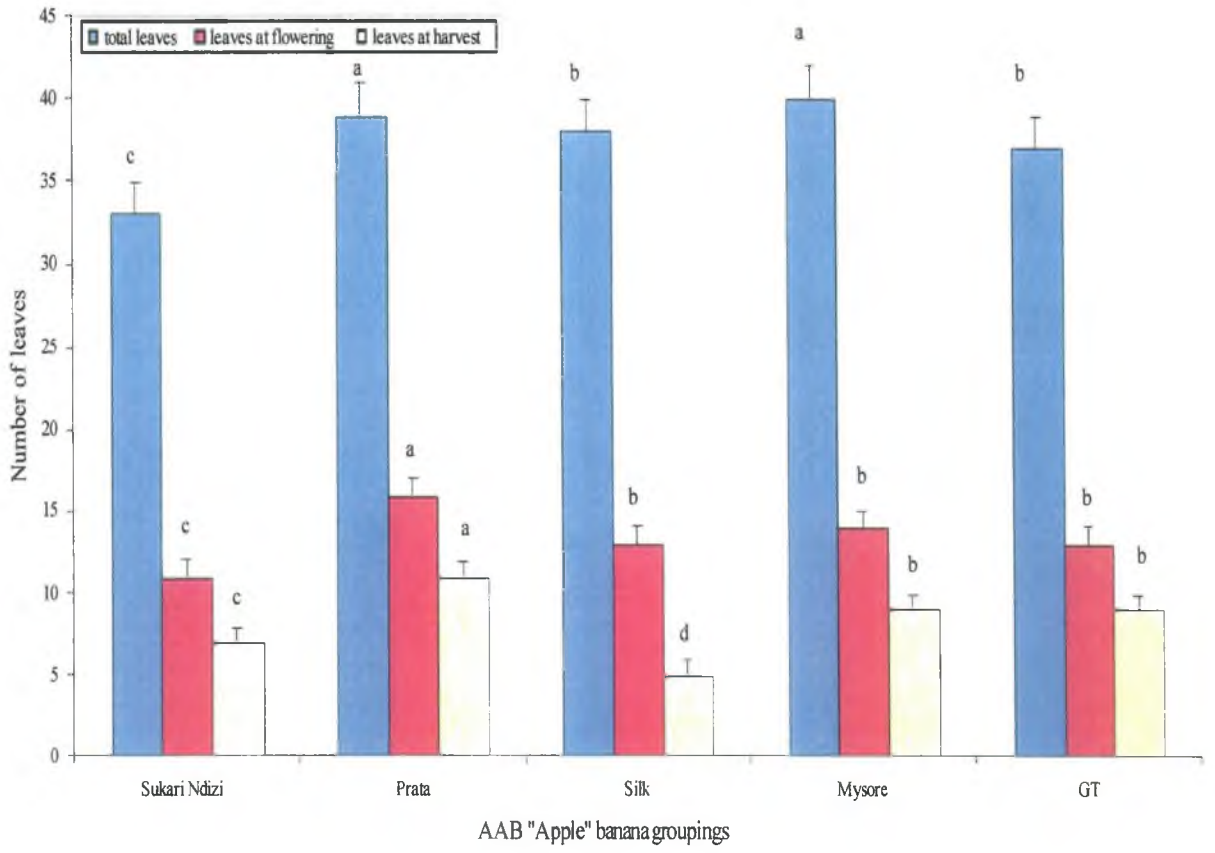


Figure 7. 5: Total leaves produced and leaves retained at flowering and at harvest for AAB "Apple" banana groupings.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

7.3.9. Total number of suckers produced by harvest time of AAB "Apple" bananas

The mean number of suckers produced by the AAB "Apple" bananas was 13, and the range was from 9 to 17 suckers. The "Sukari Ndizi" and Prata accessions produced significantly more suckers by harvest time than the Mysore and tetraploid GT. The Prata grouping were not significantly different in sucker production than the Silk grouping (Table 7.8) The Silk and Mysore groupings were not significantly

different in the total number of suckers produced by harvest time, but the Silk grouping had more suckers than the tetraploid GT. Figure 7.6 shows the monthly contribution to total sucker production of various AAB "Apple" banana accessions from the fifth month after planting.

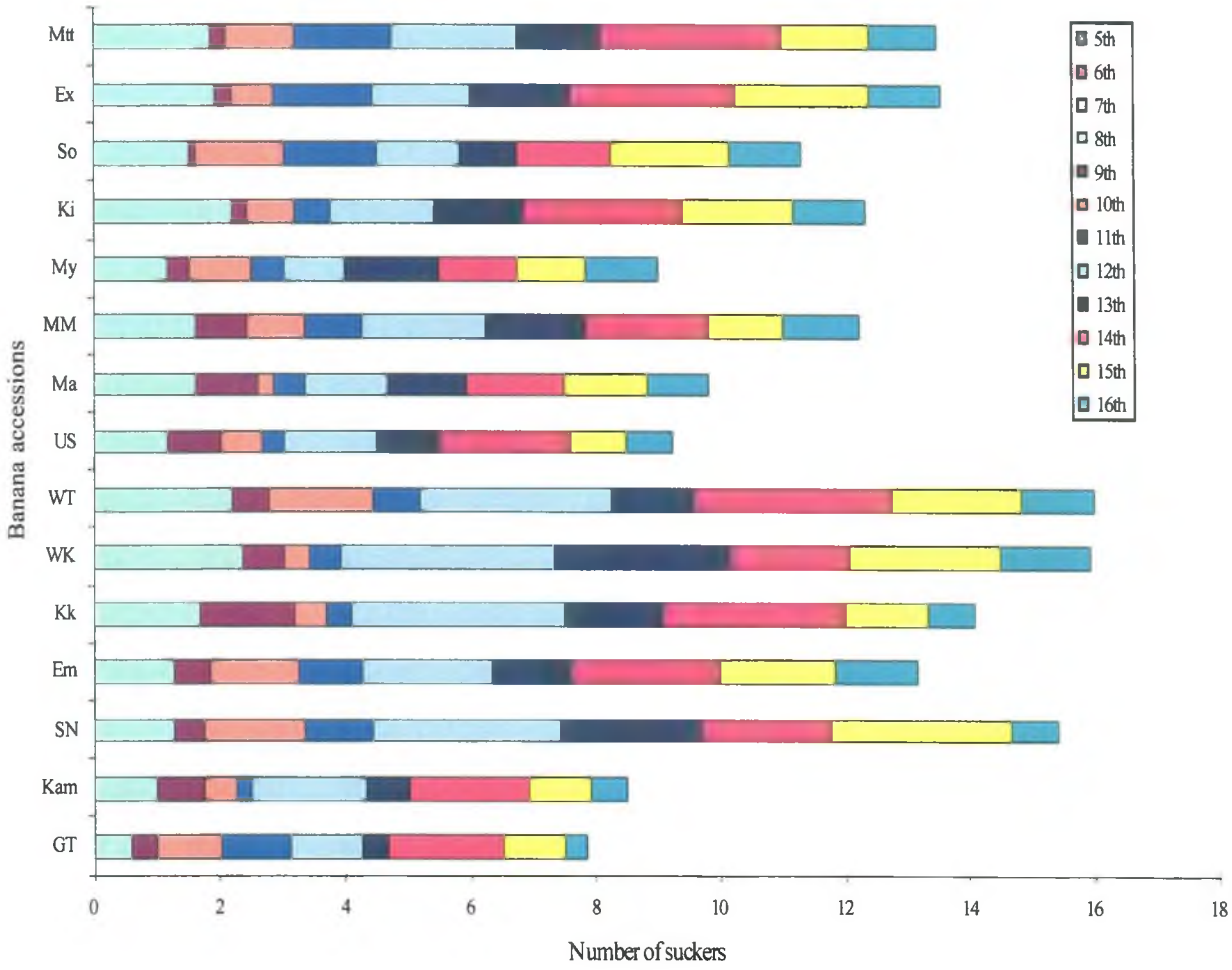


Figure 7. 6: Monthly and cumulative sucker production of AAB "Apple" dessert banana accessions.

7.3.10. Bunch weight of AAB "Apple" bananas

The mean bunch weight for AAB "Apple" bananas was 11 kilograms, and ranged from 7 to 18 kilograms. The Prata accessions had significantly larger bunches than all the other accessions. The bunch weight of the tetraploid GT and Mysore were not significantly different from each other, but were significantly greater than those of the Silk and "Sukari Ndizi" groupings (Table 7.8 and Figure 7.7). The Silk and "Sukari Ndizi" accessions had the smallest bunches, and these were not significantly different between the two groupings. However, within these groupings, the Manjano and Kamaramasenge accessions had significantly smaller bunches than Wangae Thika (Table 7.7).

Figure 7.8 shows the effect of fruit per hand and hands per bunch on the bunch weight. Increase in both fruit per hand and hands per bunch resulted in increase in bunch weight. Figure 7.9 shows the effect of leaf area and number of leaves present at harvest on bunch weight; increase in leaf area and number of leaves resulted in increase in bunch weight. Figure 7.10 shows the effect of fruit weight and number per bunch on the bunch weight of the various "Apple" banana groupings. Increase in fruit weight results in increase in bunch weight. For the Mysore, however, increase in fruit number produced more increase in bunch weight, compensating for the low fruit weight.

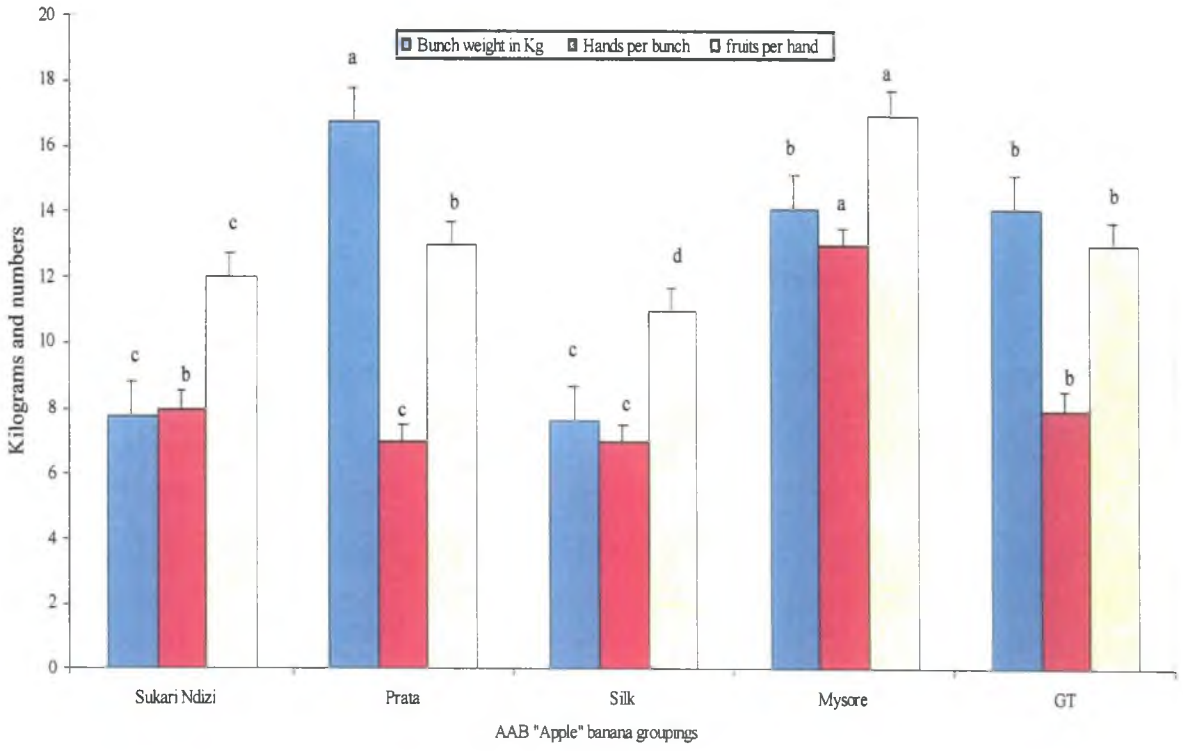


Figure 7. 7: Bunch weight, number of hands per bunch and fruits per hand of AAB "Apple" banana groupings.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

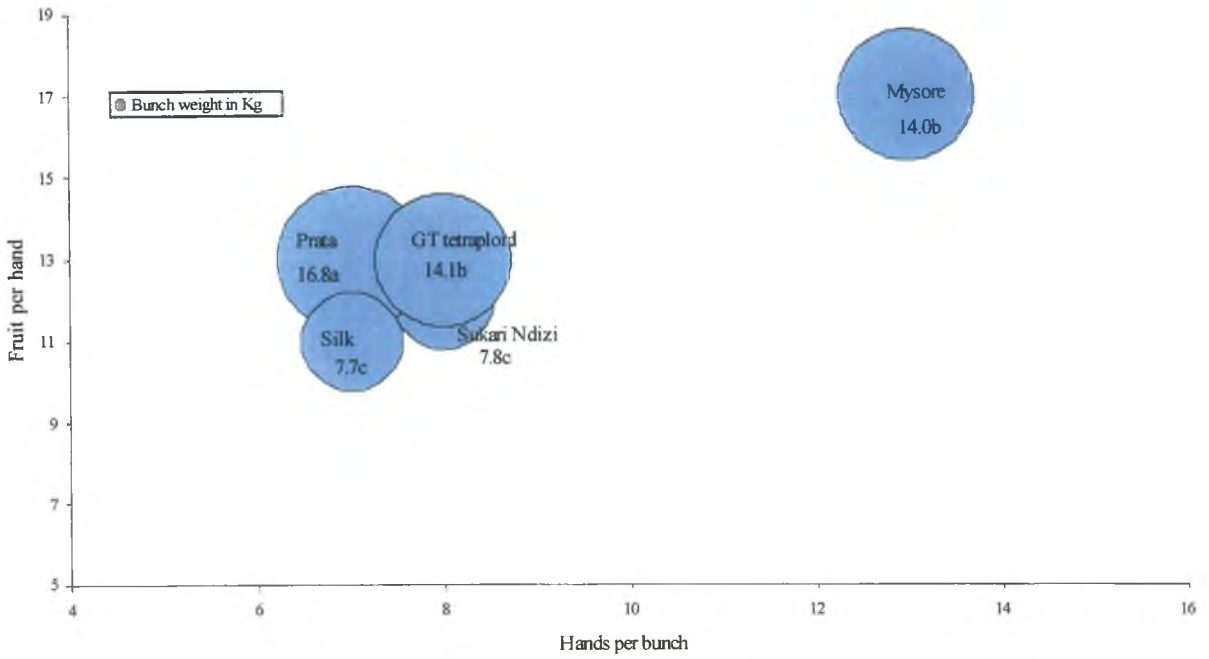


Figure 7. 8: Effect of hands per bunch and fruit per hand on bunch weight of AAB "Apple" banana groupings.
Values followed by the same letter/s coding are not significantly different among bubbles according to DMRT at $p \leq 0.05$.

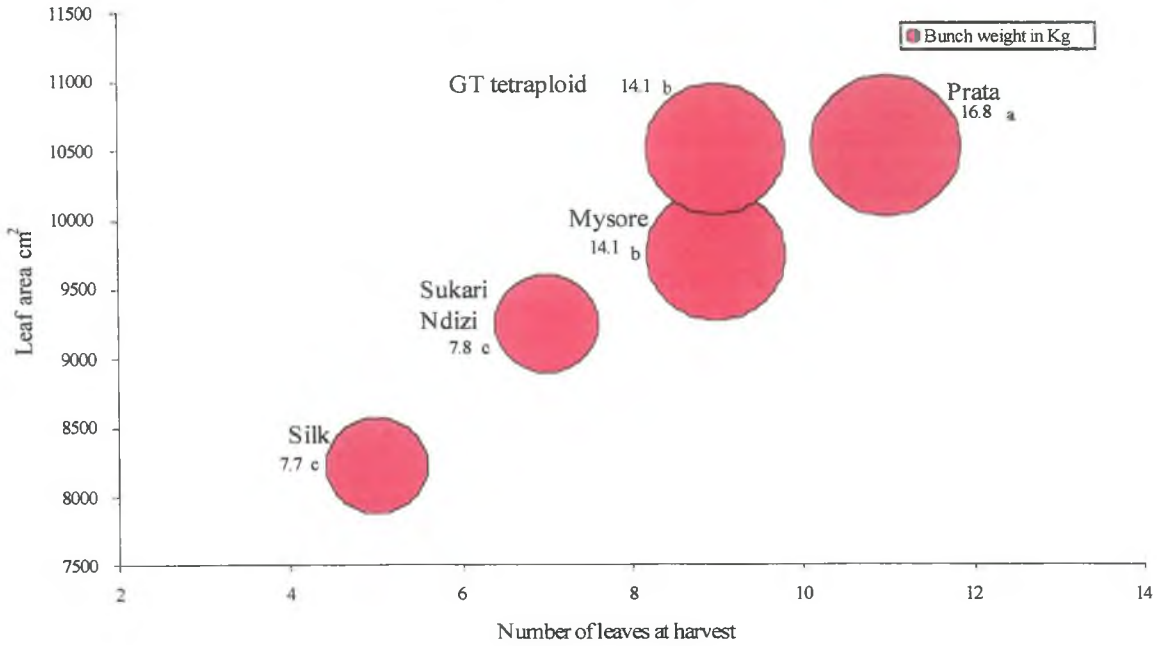


Figure 7. 9: Effect of leaf area and number of leaves at harvest on bunch weight of AAB "Apple" banana groupings.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

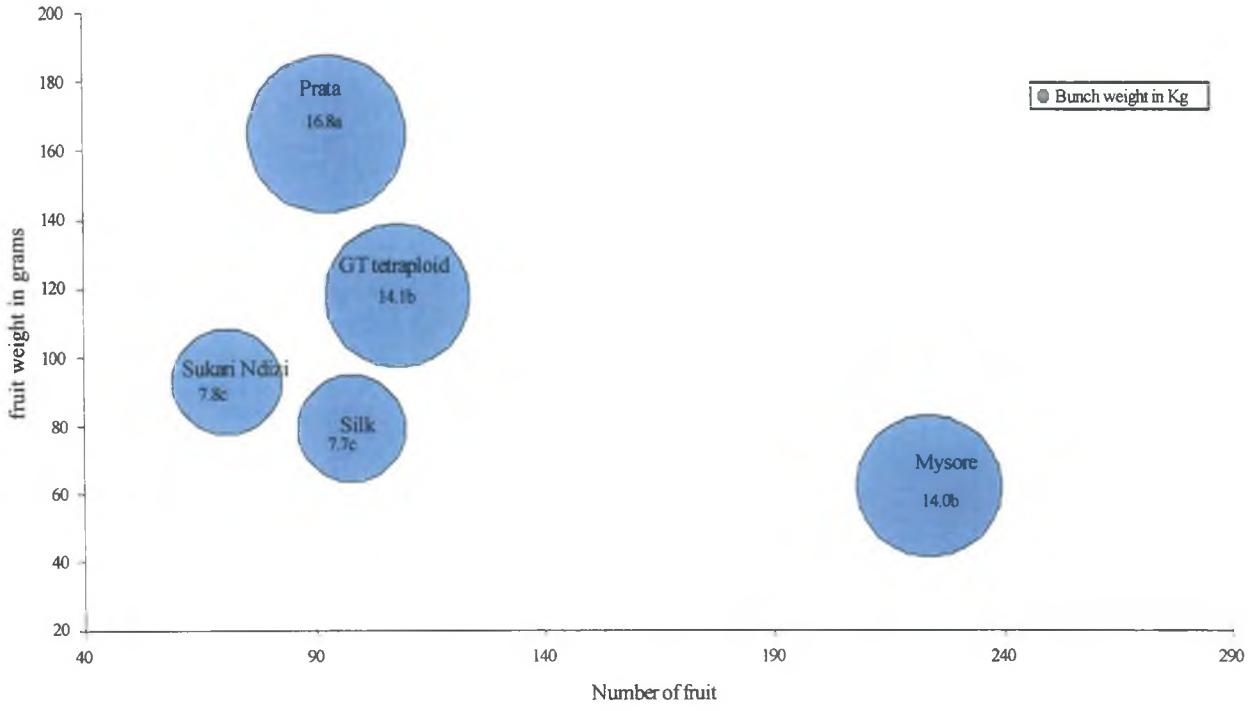


Figure 7. 10: Effect of fruits weight and number on bunch weight of AAB "Apple" banana groupings.
Values followed by the same letter/s coding are not significantly different among bubbles according to DMRT at $p \leq 0.05$.

7.3.11. Number of hands per bunch of AAB "Apple" bananas

The mean number of hands per bunch for AAB "Apple" bananas was 8, and the range among accessions from 6 to 13 hands. The Mysore had significantly more hands than all of the other accessions (Tables 7.7 and 7.8, and Figures 7.7 and 7.8). The tetraploid GT and the "Sukari Ndizi" groupings, except for the Kamaramasenge (Table 7.7), had significantly more hands than the Prata and Silk accessions. The Prata and Silk groupings were not significantly different in number of hands, except for the Ungoye Sweet of the Silk grouping that had significantly fewer hands than the Kifutu of the Prata grouping.

7.3.12. Number of fruit per hand of AAB "Apple" bananas

The mean number of fruits per hand for "Apple" bananas was 13 and the range in the number of fruit per hands was from 10 to 18. The Mysore had significantly more fruit per hand than all of the other accessions, while there was no significant difference between the Prata grouping and the tetraploid GT. The "Sukari Ndizi" grouping had significantly more fruit per hand than the Silk grouping (Tables 7.7 and 7.8, and Figures 7.7 and 7.8). The Silk had significantly fewer fruit per hand compared to other "Apple" accessions, except for the Kamaramasenge accession (Table 7.7).

7.3.13. Number of fruit per bunch of AAB "Apple" bananas

The mean number of fruit per bunch for AAB "Apple" bananas was 100, and the range among accessions was from 70 to 225 fruits. The Mysore had significantly more fruit per bunch than all of the other accessions (Table 7.7 and 7.8, and Figure 7.10). The tetraploid GT had significantly more fruit per bunch than the Prata, "Sukari Ndizi" and Silk groupings. The Prata and "Sukari Ndizi" groupings were not significantly different in total number of fruit per bunch. The Silk grouping had significantly fewer fruit per bunch than the other groupings (Table 7.8) but was not different from the Kamaramasenge accession of the "Sukari Ndizi" grouping.

7.3.14. Hand weight of AAB "Apple" bananas

The mean hand weight for "Apple" bananas was 1.342 kg, with a range from 0.833 to 2.296 kg among accessions. The clones of the Prata grouping had significantly greater hand weight than any of the other accessions (Table 7.8). The tetraploid GT had a significantly greater hand weight than the remaining AAB "Apple" accessions. The Silk had significantly greater hand weight than the "Sukari Ndizi" grouping and the Mysore. The "Sukari Ndizi" had significantly greater hand weight than the Mysore.

7.3.15. Single fruit weight of AAB "Apple" bananas

The mean fruit weight for "Apple" bananas was 106 grams; the range in fruit weight was 62 to 175 grams among accessions. Here again the Prata accessions had significantly greater fruit weight than all the other accessions. The tetraploid GT and the Ungoye sweet of the Silk group were not significantly different in fruit weight; however on average the GT had significantly greater fruit weight than the Silk and "Sukari Ndizi" groupings and the Mysore. The Silk grouping in turn had greater fruit weight than the "Sukari Ndizi" grouping and the Mysore. The Mysore fruit were significantly lighter than those of all the other "Apple" accessions. Increase in fruit weight resulted in increase of bunch weight (Figure 7.10) in most AAB "Apple" bananas. The Mysore bananas had many fruits that compensated for the small fruit weight.

7.3.16. Length of fruit of AAB "Apple" bananas

The mean length of fruit for "Apple" bananas was 16.7 centimeters. The range in fruit length was 13 to 22 centimeters among accessions. The Prata accessions had significantly longer fruit than the other "Apple" groupings (Table 7.7 and 7.8 and Figure 7.11). The Silk grouping had significantly longer fruit than the Mysore and the "Sukari Ndizi" grouping. The tetraploid GT had significantly longer fruit than the "Sukari Ndizi" grouping and the Mysore also had significantly longer fruit than the "Sukari Ndizi" grouping.

7.3.17. Circumference of fruit of AAB "Apple" bananas

The mean circumference of fruit for "Apple" bananas was 12.8 centimeters; the range in fruit circumference was 11 to 15 centimeters among accessions. The Prata accessions and the tetraploid GT were significantly larger in this dimension than the other AAB "Apple" accessions (Table 7.7 and 7.8 and Figure 7.11). There was no significant difference in fruit circumferences between the "Sukari Ndizi" and the Silk groupings, but the Mysore grouping had significantly smaller finger circumference than the rest of the "Apple" bananas.

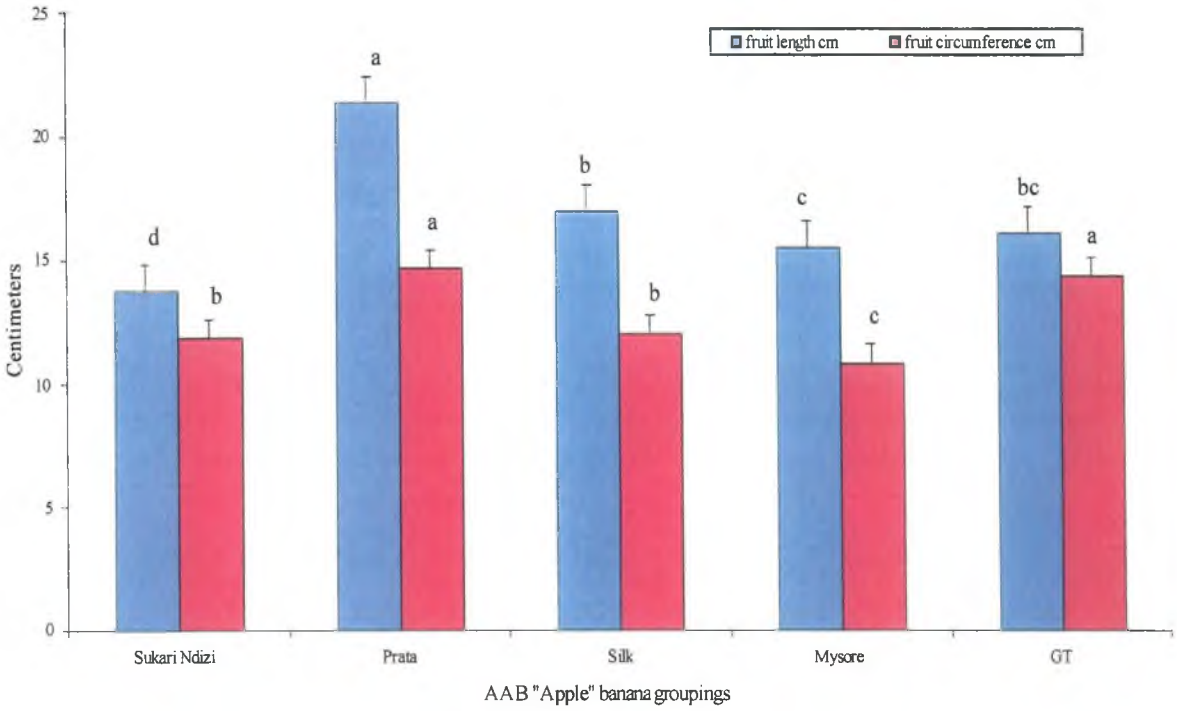


Figure 7. 11: Comparison of fruit length and circumference of AAB "Apple" dessert banana groupings.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

7.3.18. Yield potential of AAB "Apple" bananas

There were significant difference in yield potential among the various AAB "Apple" groupings (Table 7.8, and Figures 7.12 and 7.13). The mean yield was 11.1 tons ha^{-1} $year^{-1}$. The Prata grouping yielded significantly higher than other AAB "Apple" followed by the tetraploid GT, then the Mysore. The Silk and "Sukari Ndizi" yielded significantly the lower than other ABB "Apple" grouping but were not significantly different from each other.

The regression equation obtained was:

$$Y = 9.8464 + 0.9719BW - 0.016DH.$$

High positive association (+0.9719) was found between the bunch weight and the yield potential. The most important component of the yield potential was the bunch weight, which was significantly associated with the yield potential in all the "Apple" banana groupings (Figures 7.12 and 7.13).

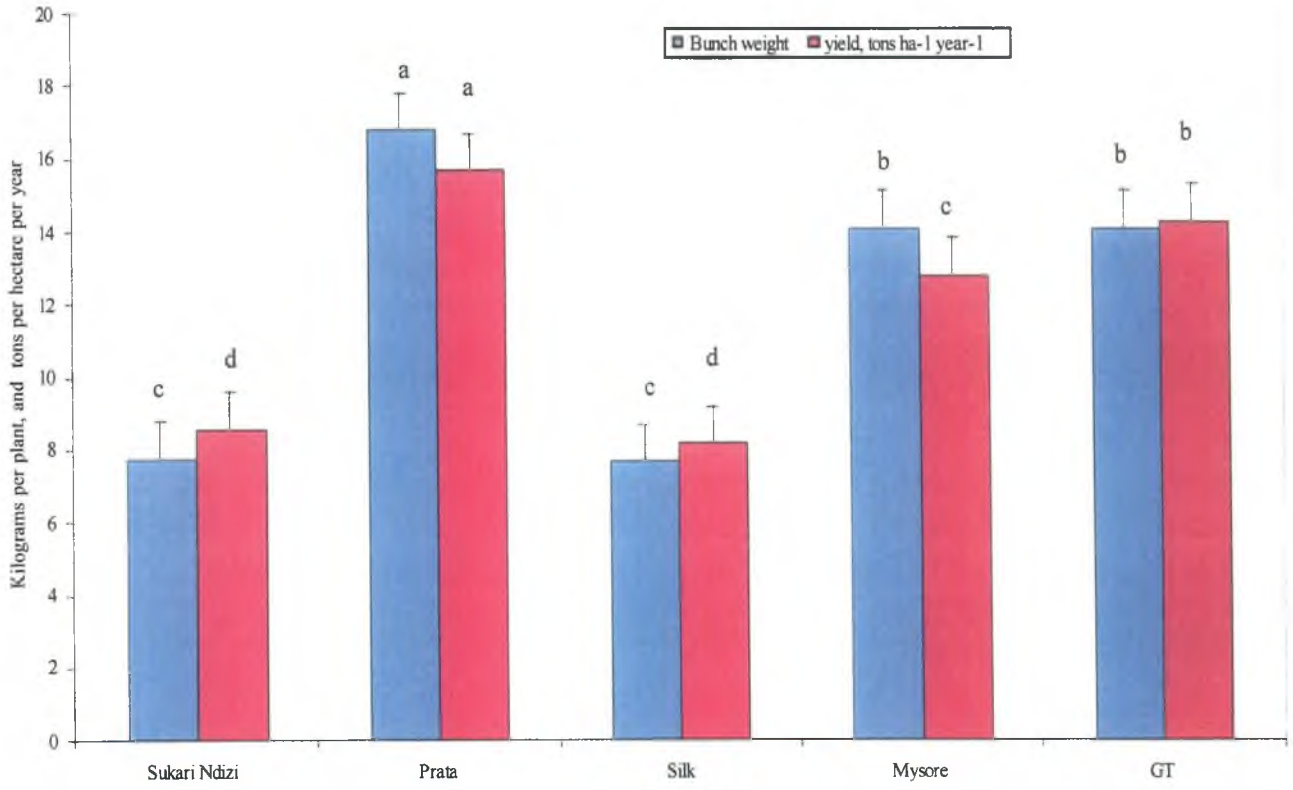


Figure 7. 12: Comparison of yield and bunch weights of AAB "Apple" dessert banana groupings.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

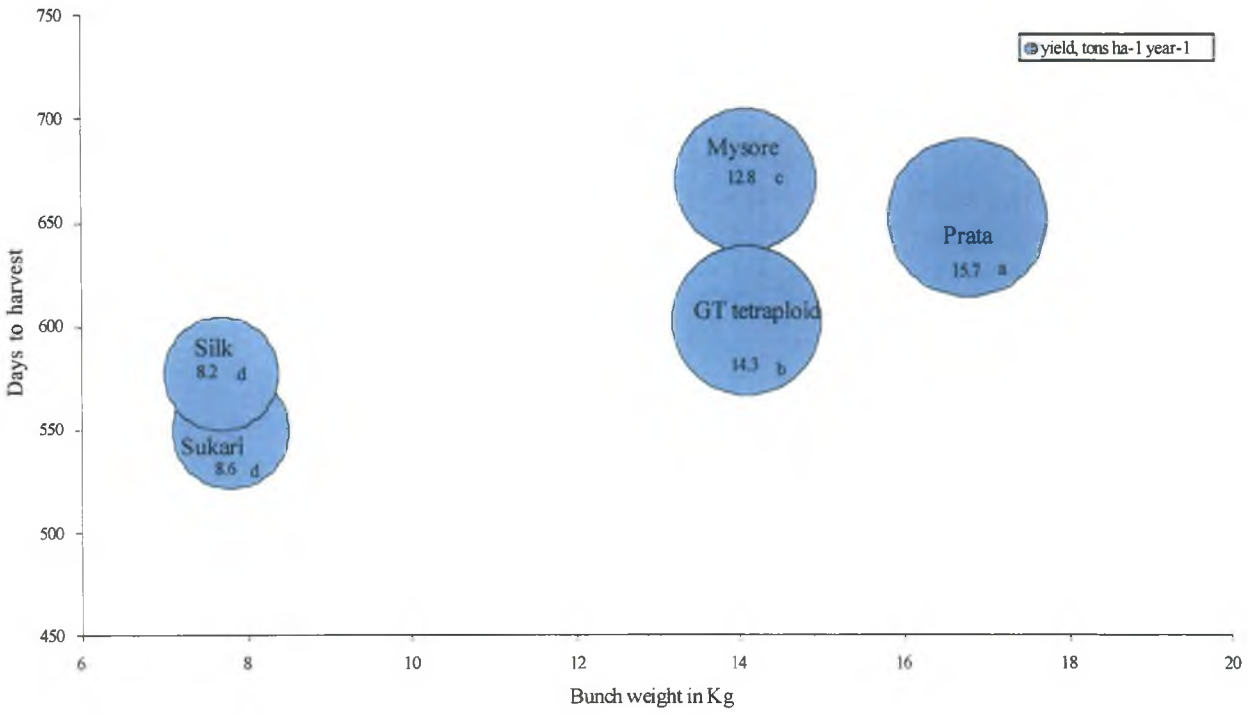


Figure 7. 13: Effect of bunch weight and days to harvest on potential yield tons ha^{-1} $year^{-1}$ of AAB "Apple" dessert banana groupings
Values followed by the same letter/s coding are not significantly different among bubbles according to DMRT at $p \leq 0.05$.

7.4. Results of AA "Muraru" banana trial

There were significant differences among the AA "Muraru" accessions in days from planting to flowering, days from planting to harvest, and days from flowering to harvest (Table 7.9 and Figure 7.14). Significant differences occurred among the AA "Muraru" accessions in plant height at flowering, girth of plants at flowering, and leaf area (Table 7.9 and Figure 7.15). There were significant differences among the "Muraru" accessions on bunch weight, hand weight, fruit weight, length and circumference of fruit, and potential yield (Table 7.10). No significant differences were found among the "Muraru" accessions in other variables (Table 11), i.e., number of hands per bunch (7), number of fruit per hand (13), mean fruit per bunch (90), total suckers produced by harvest (6), total number of leaves (47), number of leaves at flowering (16), and number of leaves at harvest (11) (The means of each variable for the "Muraru" accessions are given in parenthesis and in Table 11).

7.4.1. Days to flower of AA "Muraru" bananas

There were significant differences in days from planting to flowering among the AA "Muraru" accessions. The mean number of days to flowering was 513, and the range was 473 to 544 days (Table 7.9). Makyughu, from Arusha, Tanzania, took significantly longer to flower than the Kamunyilya, Njuru, TT2, Majimaji, and Muraru Red-bell, but not significantly longer than the other Muraru accessions (Figure 7.14). Kamunyilya took significantly fewer days to flower than any of the other "Muraru" accessions, an indication that it was horticulturally superior in this

respect. The AA "Muraru" accessions took a mean of 513 days to flowering, compared with the AAB "Apple" accessions which took a mean of only 422 days.

7.4.2. Days from planting to harvest, and days between flowering and harvest for AA "Muraru" bananas

The Muraru cultivars had significant differences in the number of days from planting to harvest. Makyughu took significantly longer to produce a harvestable bunch than all other "Muraru" accessions, while Njuru and Kamunyilya took significantly less time to produced harvestable fruits than other "Muraru" accessions, with the exception of TT2 and Mraru Mlalu accessions (Table 7.9). The mean number of days to harvest for the "Muraru" accessions was 678 days, and the range was from 655 to 710 days.

Kamunyilya accession took significantly longer from flowering to harvest than Njuru, Muraru Mshare, and Mraru Mlalu accessions, but was not significantly different from the other "Muraru" accessions. The Mraru Mlalu accession took significantly less time from flowering to harvest than any to all other "Muraru" accession (Table 7.9).

7.4.3. Plant height and girth at flowering of AA "Muraru" bananas

The mean plant height of the "Muraru" banana accessions was 3.07 m; the range was from 2.8 to 3.3 m. The Muraru Mshare, Muraru, TT2, and Muraru red bell were significantly taller than Njuru, Kamunyilya, Makyughu, Maji maji, and Muraru green bell accessions (Table 7.9 and Figure 7.15).

The TT2, Muraru, Muraru Mshare, Muraru red bell accessions had significantly greater plant girth than Mraru Mlalu, Muraru green bell, Kamunyilya, Makyughu and Njuru accessions. Majimaji accession had medium girth (Table 7.9 and Figure 7.15).

Table 7. 9: Analysis of vegetative traits of the *Musa* AA "Muraru" banana accessions grown in Kisii, Kenya

Accession	**Days to flowering**	**Days to harvest**	**Flower to harvest**	**Girth (cm)**	**Plant height (meters)**	**Leaf area (cm^2)**
TT2	497^{c}	665^{cd}	168abc	52.4a	3.22^{ab}	9825^{b}
Muraru	524^{ab}	693^{b}	168abc	53.1a	3.17^{ab}	9735^{b}
Mraru Mlalu	525^{ab}	666^{cd}	141d	50.1b	3.11^{bc}	9683^{b}
Muraru Mshare	526^{ab}	679^{bc}	153cd	53.4a	3.26^{a}	9437^{b}
Njuru	497^{c}	661^{d}	163bc	47.6c	2.85^{d}	8377^{c}
Muraru green bell	525^{ab}	691^{b}	166abc	49.9b	3.03^{c}	9170^{bc}
Muraru red bell	517^{bc}	683^{b}	166abc	52.8a	3.17^{ab}	9422^{b}
Kamunyilya	473^{d}	655^{d}	181a	49.6b	3.02^{c}	8990^{bc}
Maji maji	505^{bc}	681^{bc}	176ab	51.5ab	3.03^{c}	9428^{b}
Makyughu	544^{a}	710^{a}	165abc	49.8b	2.82^{d}	10946^{a}
Critical range	24.23	18.22	17.36	2.2	0.1462	1398
Mean	513	678	165	51	3.07	9491
CV	4.9%	2.8%	11%	4.5%	5 %	10.5%

Means in the same column with the same letter/s coding are not significantly different, according to DMRT at $p \leq 0.05$.

7.4.4. Leaf area of AA "Muraru" banana accessions

The Makyughu accession had significantly greater leaf area than any other "Muraru" accession, while the Njuru accession had the least leaf area, but was not significantly different from Kamunyilya or Muraru green bell accessions (Table 7.9).

7.4.5. Hand weight, fruit length and circumference of AA "Muraru" bananas

Muraru and Majimaji had significantly heavier hands than all the other "Muraru" accessions (Table 7.10). Muraru green bell had the smallest hand weight, but it was not significantly different from Makyughu or Muraru Mlalu (Table 7.10 and Figure 7.17).

Muraru Mshale had significantly longer fruits than any other "Muraru" accessions except the Muraru accession. Muraru had significantly longer fruits than any of the remaining "Muraru" accessions except the TT2 and Muraru Mlalu accessions. The Njuru accession had significantly shorter fruits than any other "Muraru" accession (Table 7.9 and Figure 7.16).

The fruit circumferences of the Kamunyilya accession were significantly larger than all other "Muraru" accessions except for the Muraru Mshare, Muraru, and accessions (Table 7.9 and Figure 7.16). The fruits of Muraru red bell and Muraru Green Bell, accessions were significantly smaller in circumference than other accessions except for Makyughu and Mraru Mlalu.

Table 7. 10: Analysis of bunch weight, potential yield and fruit traits of the *Musa* AA "Muraru" banana accessions grown in Kisii, Kenya

Accession	Bunch weight (Kg)	Yield (tons ha^{-1} yr^{-1})	Hand weight (grams)	Fruit weight (grams)	Fruit length (cm)	Fruit circumference (cm)
TT2	17.6^{cd}	16.1^{bc}	2159^{b}	168.5a	24.6^{b}	13.1^{bcd}
Muraru	23.7^{a}	20.7^{a}	2347^{a}	179.7a	25.6^{ab}	13.3^{abc}
Mraru Mlalu	15.8^{cd}	14.5^{bc}	1908^{cd}	151.1b	24.8^{b}	12.7^{cde}
Muraru Mshare	18.3^{c}	16.4^{b}	2165^{b}	169.3a	26.2^{a}	13.5^{ab}
Njuru	12.8^{ef}	11.8^{de}	2057^{bc}	151.6b	19.2^{e}	13.2^{abcd}
Muraru green bell	10.9^{f}	9.6^{f}	1749^{d}	133.1c	22.8^{c}	12.3^{e}
Muraru red bell	15.7^{cd}	14^{cd}	1995^{c}	149.3b	22.7^{c}	12.3^{e}
Kamunyilya	15.0^{de}	13.9^{cd}	1968^{c}	151.3b	21.2^{cd}	13.8^{a}
Maji maji	20.9^{b}	18.6^{a}	2337^{a}	171.7a	22.1^{cd}	13.1^{bcd}
Makyughu	13.1^{ef}	11.2^{ef}	1908^{cd}	149.7b	22.4^{c}	12.5^{de}
Critical range	2.85	2.455	182	16.08	1.189	0.7571
Mean	16.4	14.7	2063	157	23	13
CV	18%	17.4%	9.2%	10.6%	5.4%	6%

Means in the same column with the same letter/s coding are not significantly different, according to DMRT at $p \leq 0.05$.

Table 7. 11: The means of the traits, not significantly different among the Musa AA "Muraru" accessions grown in Kisii, Kenya

Trait	Hands per bunch	Fruit per hand	Fruit per bunch	Leaves at flowering	Leaves at harvest	Total leaves	Total suckers
Average	7	13	90	16	11	47	6

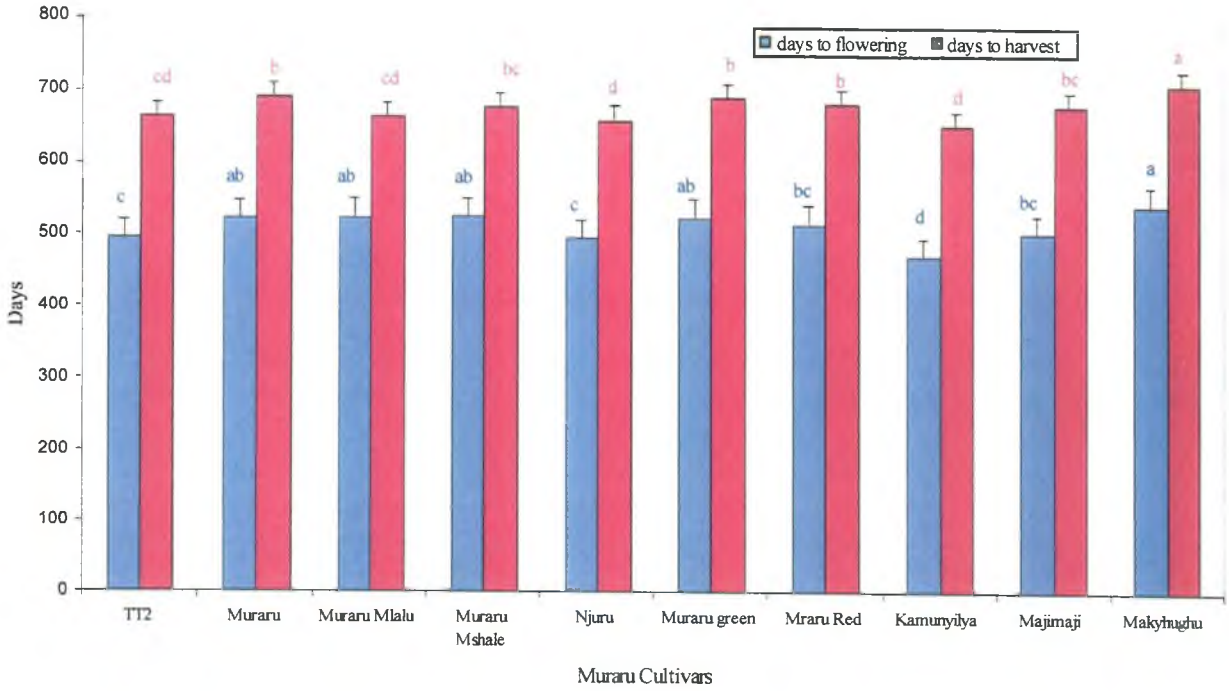


Figure 7. 14: Days from planting to flower and to harvest of *Musa* AA "Muraru" accessions.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

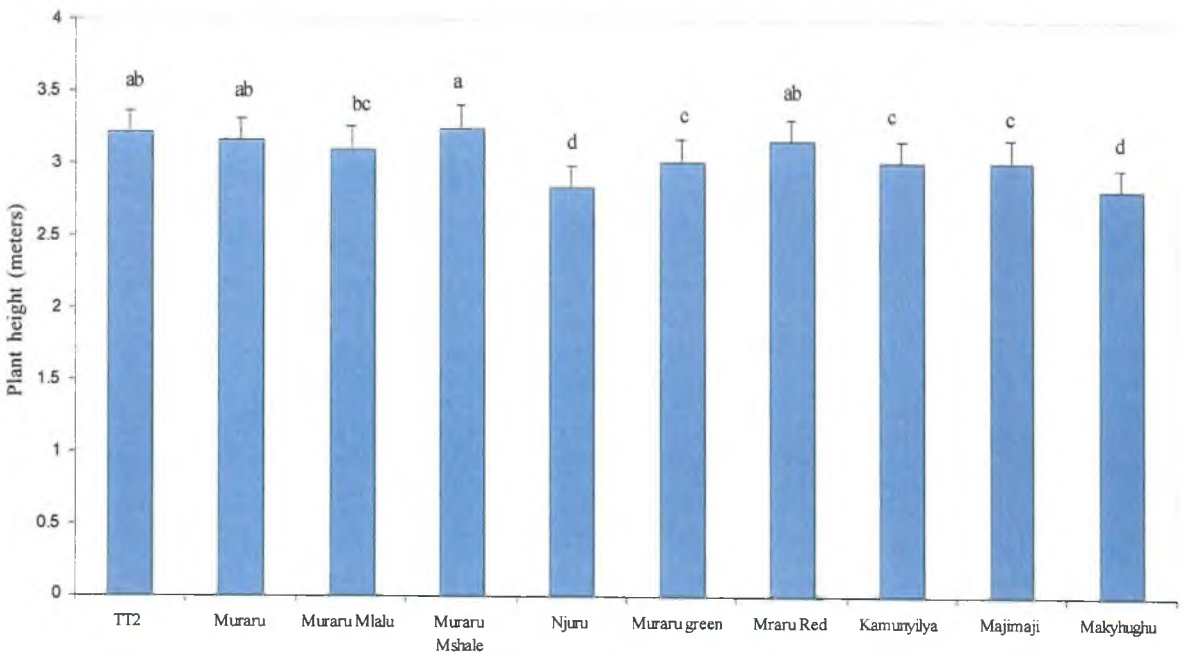


Figure 7. 15: Pseudostem height of banana accessions of AA "Muraru" accessions.
Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

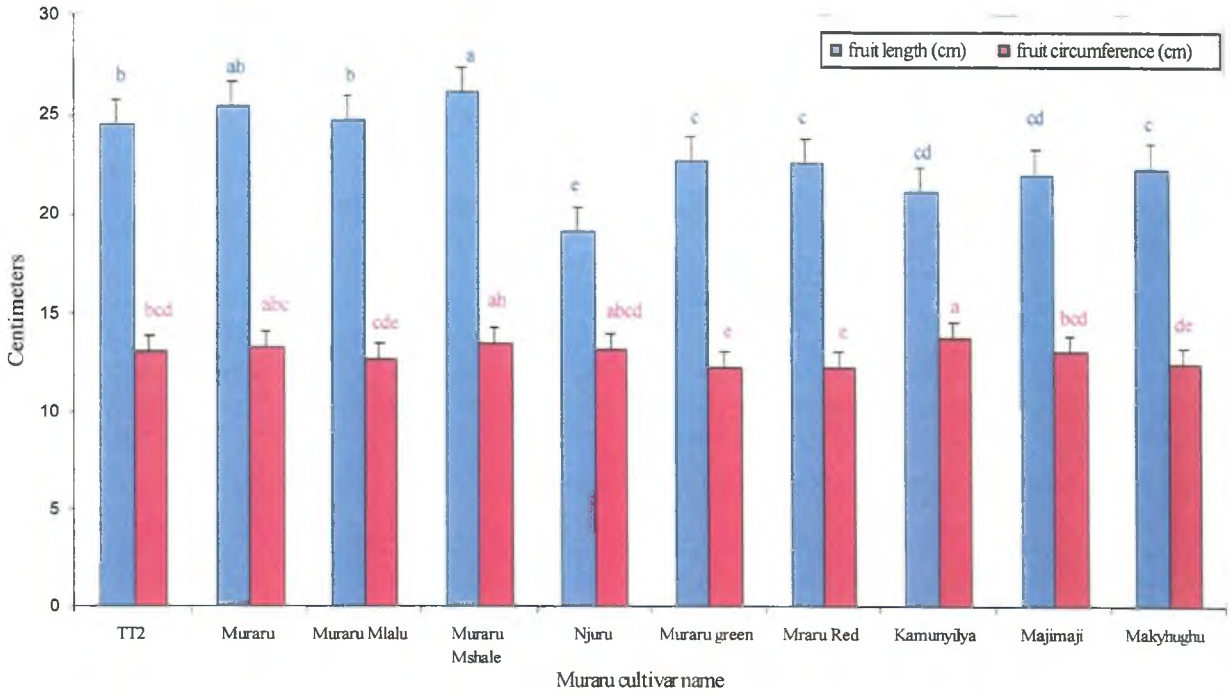


Figure 7. 16: Variation in mean fruit length and circumference of AA "Muraru" accessions. Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

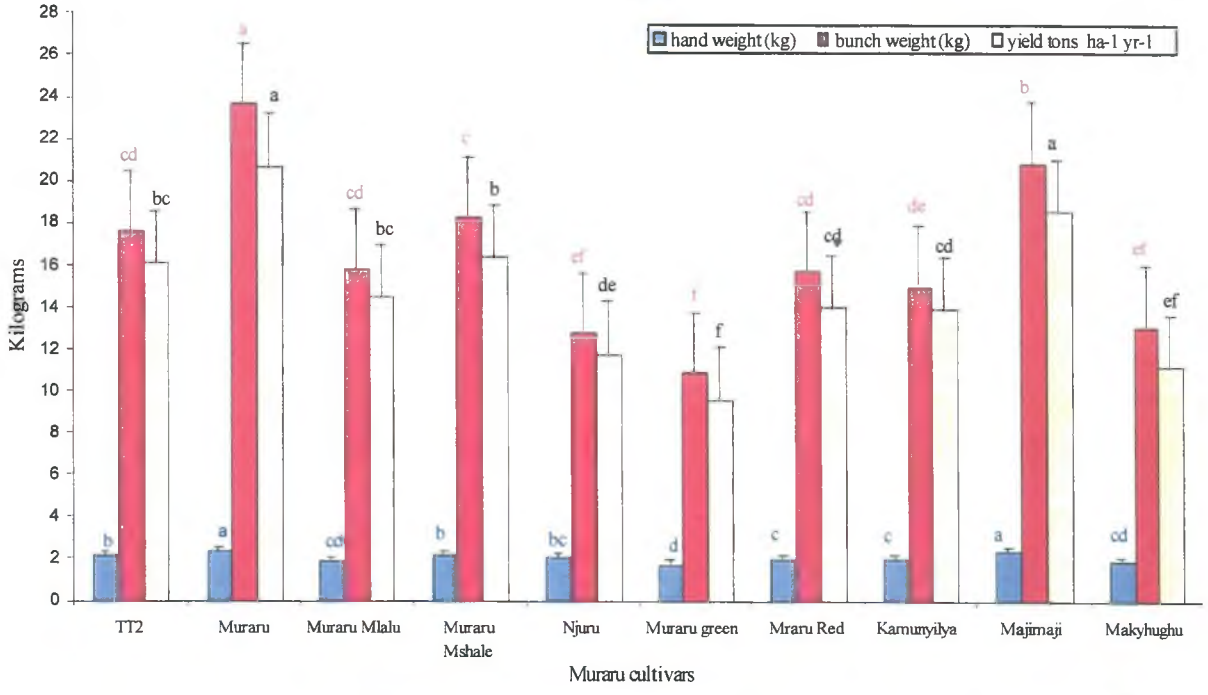


Figure 7. 17: Effect of cultivar on hand and bunch weight and yield of AA "Muraru" accessions. Values followed by the same letter/s coding for each variable are not significantly different according to DMRT at $p \leq 0.05$.

7.4.6. Bunch weight of AA "Muraru" bananas

The mean bunch weight was 16.4 kg for the "Muraru" bananas and ranged from 10.9 to 23.7 kg. The Muraru accession had significantly bigger bunches than all the other accessions (Table 7.10). The Majimaji had significantly bigger bunches than the remaining "Muraru" accessions. The bunch weights of the TT2, Mraru Mlalu, Muraru Mshale, and Muraru Red-bell were not significantly different, but were significantly bigger than those of the Muraru Green Bell, Njuru, and Makyughu (Table 7.9 and Figure 7.17).

7.4.7. Yield potential of AA "Muraru" bananas

The were significant differences in yield potential of the various "Muraru" cultivars (Table 7.10, and Figures 7.18 and 7.19). The mean yield was 14.7 t ha^{-1} $year^{-1}$. The regression analysis showed a high positive association between bunch weight and yield.

The regression equation obtained was:

Yield = 13.979 + 0.8741BW – 0.0201DH.

A high positive association (+0.8741) existed between bunch weight and yield potential. The bunch weight, which was significantly associated with yield potential in all the "Muraru" accessions, was the most important yield component (Figures 7.18 and 7.19).

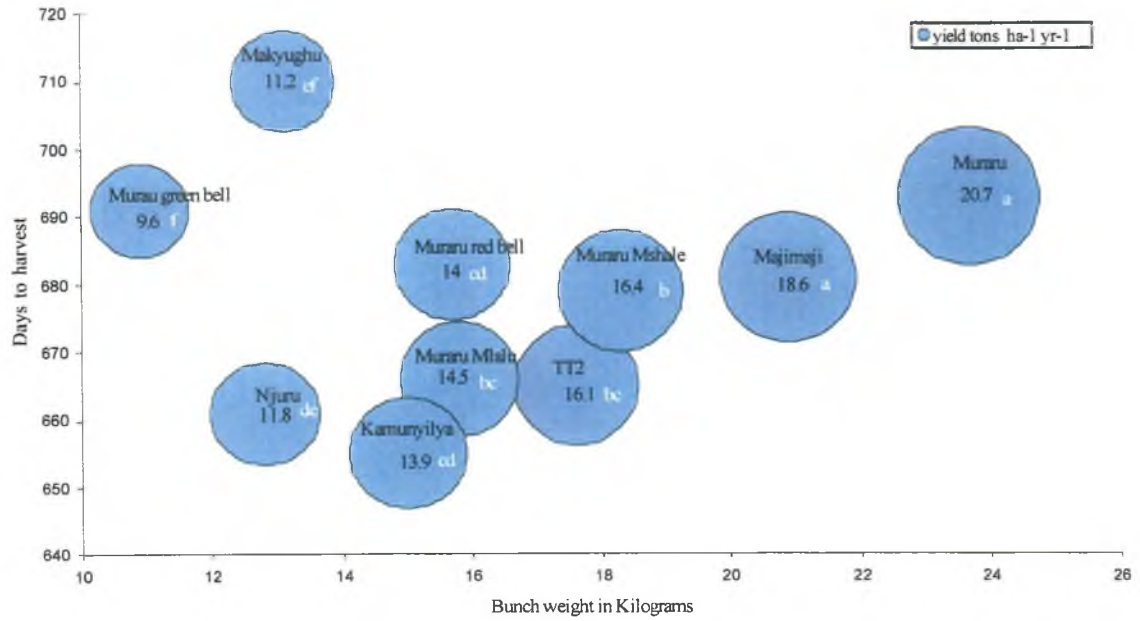


Figure 7. 18: Effect of bunch weight and days to harvest on potential yield in tons ha^{-1} year^{-1} . Values followed by the same letter/s coding are not significantly different among bubbles according to DMRT at p≤ 0.05.

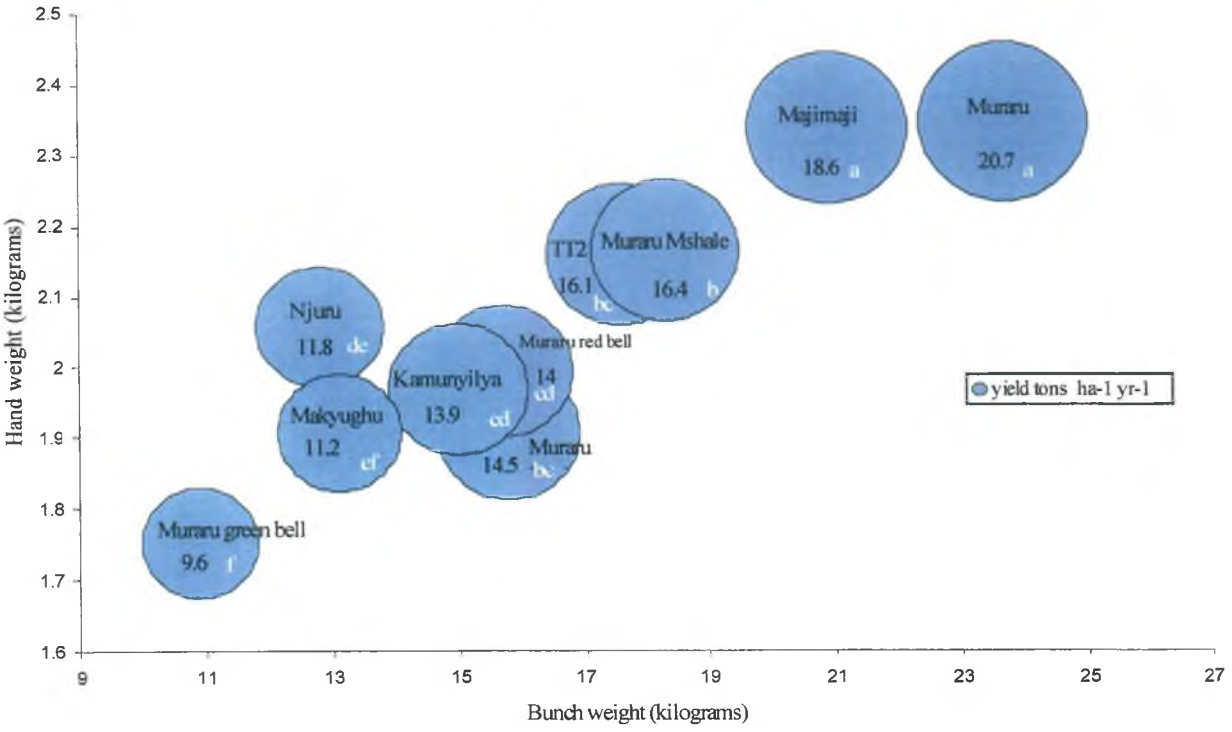


Figure 7. 19: Effect of bunch and hand weight on yield of AA "Muraru" accession. Values followed by the same letter/s coding are not significantly different among bubbles according to DMRT at p≤ 0.05.

7.5. Discussion of AAB "Apple" and AA "Muraru" banana trials

7.5.1. Statistical Analysis of horticultural traits variation

The ANOVA found significant differences among the AAB "Apple" dessert banana groupings including the tetraploid GT for all the 19 horticultural traits tested. These differences can be attributed to genetic differences among the clones. The Duncan's multiple range mean separation based on groupings from the phenograms of microsatellite markers showed distinct differences among the groupings (the Mysore, Prata, Silk, and "Sukari Ndizi"). This is what we would expect, because the grouping relationships were initially named in recognition of shared morphological similarities of the member clones. In a separate study (chapter 4 and 5), microsatellite markers separated these four AAB "Apple" groupings, and included the tetraploid GT clone within the "Sukari Ndizi" grouping. Based on these horticultural traits, the seedling-derived tetraploid GT clone was not obviously a member of any of the other groupings and forms a distinct category horticulturally.

For many traits, Kamaramasenge accession was an "under-performer," compared to the other "Sukari Ndizi" clones, and it appeared not to belong in this grouping horticulturally. However, that discrepancy was likely the result of Panama wilt, which affected this clone more than others. Molecular data, using microsatellite markers, clearly show the Kamaramasenge as a "Sukari Ndizi" clone.

Nine of the 19 tarits evaluated by ANOVA in the diploid AA "Muraru" clones found no significant variation among the accessions. The lack of differences among the "Muraru" accessions in these traits is probably an indication of a narrow diversity, which was consistent with the fact that these were all considered members of a single

grouping of genetically related clones based on microsatellite marker analysis, with the exception of Kamunyilya. There were, however, ten important traits including days to flowering, days between flowering and harvest, days to harvest, plant height and girth, bunch weight, hand weight, fruit length and circumference, and potential yield that had significant variation among the "Muraru" accessions (Tables 7.9 and 7.10). In contrast to the situation with Kamaramasenge in the AAB "Apple" "Sukari Ndizi" grouping, Kamunyilya was probably of a different AA genotype than its "Muraru" cohorts, based on molecular data, yet most of its horticultural characteristics seemed consistent with the AA "Muraru" grouping.

7.5.2. Clone evaluation for horticultural merit

The most important horticultural characteristics for field evaluation of clone performance were days to harvest and bunch weight. As expected, the bunch weights for both the AAB "Apple" and the AA "Muraru" accessions were positively correlated to potential yields and the days to harvest were negatively correlated to the potential yields. Based on the potential yields, the Prata grouping in general, particularly the Soth accession, and the GT were most productive among the AAB "Apple" clones, as were the Muraru and Majimaji accessions among the "Muraru" clones.

The mean bunch weight was 11 kilograms for the AAB "Apple" bananas. Individual fruit weight, generally, was significantly and positively correlated with bunch weight, except for Mysore, which had small fruit; Mysore's bunch weight was influenced more by fruit number than fruit weight (Figure 7.10). Hands per bunch

and fruit per hand were not major factors in determining the bunch weight for most cultivars, as indicated in Figure 7.8. For example, although Mysore had the most hands and most fruit per hand, the size of the bunch was not the largest. The number of leaves still present at harvest was positively correlated with the bunch weight (Figure 7.9), and could explain why the Silk accessions had a low bunch weight. All three Silk accessions lost most leaves by harvest time, due to Panama disease.

The mean bunch weight was 16.4 kg for the "Muraru" bananas; Muraru and Majimaji accessions would be preferred for big bunch. Muraru Green Bell had a small bunch and was late maturing, making it less desirable.

In the Kisii environment, bananas generally took longer to harvest compared to other banana growing areas (Stover 1987). The mean number of days to harvest was 594 for the AAB "Apple" bananas. Generally, the accessions that took longer to flower also took long to reach the harvest stage. The "Sukari Ndizi" and Silk groupings were horticulturally superior with respect to time required to reach maturity; as it took these accessions less time to develop to the harvestable stage.

The mean of 678 days from planting to harvest for "Muraru" accessions was very long compared to published reports of other banana groups that take between 301 and 473 days to harvest (Stover 1987, Ortiz and Vuylsteke 1998). Among the "Muraru" accessions, Kamunyilya, Njuru, TT2, and Muraru Mlalu cultivars were earliest to harvest and are superior in this regard.

Yield potential results obtained were similar to those of Ortiz and Vuylsteke (1998). Days to harvest was negatively associated (-0.016) with the yield potential of the AAB "Apple" bananas in this study; the annual productivity of clones was

reduced as days to harvest increased. Because of the long development period at Kisii, there was a reduction in potential yield for the AAB "Apple" bananas. The tetraploid GT accession had a shorter crop cycle than the Mysore, and as a result, the yield potential was significantly higher than that of Mysore, although their bunch weights were not significantly different. The high bunch weight of the Prata accessions compensated for their long growing cycle, giving them a better yield potential, followed by the GT.

For the "Muraru" accessions, the regression analysis also showed a high positive association between bunch weight and yield potential. Similar yield potential results were obtained by Ortiz and Vuylsteke (1998), using various banana cultivars. Days from flowering to harvest was negatively (-0.0201) related to the yield potential of the "Muraru" cultivars in this study. There was reduction in the potential yield for cultivars with long growing cycles. As bunch weight increased, yield also increased, and as hand weight increased, yield also increased. However when the number of days taken to harvest increased, the cultivars' annual yields decreased, compared to those with similar bunch weights, but faster maturity.

7.5.3. Finger dimensions

A long fruit is desirable because this trait is used to grade commercial banana fruit (Stover 1987). Among the AAB "Apple" dessert bananas, the Prata and Silk accessions and the GT were superior in this respect. Most of the "Muraru" accessions had long fruits, although it was possible to still divide the "Muraru" accessions into two sets: those with longer fruit (TT2, Muraru Mshale, and Muraru Mlalu), and

therefore, probably more desirable, and those with shorter fruit (Njuru, Kamunyilya, Makyughu and Muraru Red Bell). Njuru had the shortest fruit making it an inferior accession, based on this trait. In some studies (Stover 1987), it was found that finger length increases rapidly in the first 30 days, then slows and the increase is complete in 40 to 80 days, while the finger diameter increases until harvest, an indication that finger length is probably a better trait than diameter for cultivar identification. The mean finger length for Cavendish bananas, for example, is 23.6cm for the ratoon crop and the ranges from 21.9 to 25.3 cm (Robinson *et al.* 1993).

Shorter banana plants are generally preferred for ease of harvesting, stability in windy areas, and other operations in the orchard. The significant variation in pseudostem height among "Apple" groupings was evidence of genetic diversity within the AAB "Apple" group of dessert bananas. The AAB "Sukari Ndizi" and Silk subgroups were horticulturally superior in this respect, as they were short, and this character is useful in windy areas to avoid lodging. The tetraploid GT was also superior in this regard, compared to Mysore and Prata accessions that were tall.

The "Muraru" accessions had a higher mean height of 3.07 meters. The Muraru Mshale, Muraru Mlalu, TT2, and Muraru were the tall clones while the Njuru, Makyughu, and Kamunyilya were the relatively shorter clones. The other clones had medium height. The variation in pseudostem height was, however, small.

While the mean plant girth of "Apple" banana accessions was 47.5 centimeters, the tetraploid GT and Prata accessions were superior based on this trait. Their pseudostem was stouter and, therefore, stronger in windy conditions, compared to the Mysore, Silk and "Sukari Ndizi" groupings.

7.5.4. Effect of study site climate on banana performance

In general, the growth rates observed at Kisii, Kenya, were rather slow, compared with rates recorded elsewhere. The time required for AAB "Apple" bananas from planting to flowering is climate dependent too; it takes less time in warmer areas than in cooler areas, because the plant growth rate increases with increasing temperatures. In Kisii, the earliest "Apple" banana accession flowered at 362 days. According to Stover (1987), in the Honduras, the banana plants (the cultivar is not mentioned) began to flower 180-210 days after planting, although this period could extend to 250 days. The generally long vegetative period in Kisii was due to lower temperatures experienced (Figures 7.2 and 7.3). The data from this study indicated that the time from planting to flowering was probably also dependent on the genetic composition of the plant. Accessions within the same group generally flowered at around the same time, with the "Sukari Ndizi" grouping requiring the shortest time and Mysore, the longest.

The days to flowering for the "Muraru" accessions were many, compared to those of the AAB "Apple" bananas grown in the same environment during the same period. Compared to other banana cultivars such as plantains, Cavendish, Gros Michel (Ortiz and Vuylsteke 1998), the AA "Muraru" took longer to flower. The planting to flowering time of the "Muraru" was dependent on the genotype as well as environmental influence. While in Kisii, it generally took longer for bananas to flower, in Onne, Nigeria (Ortiz and Vuylsteke 1998), the average flowering time for all banana plants was 258 days. The generally long duration taken to flowering in Kisii is due to the lower temperatures experienced (Figures 7.2 and 7.3). The AA

"Muraru" accessions took more days to flower compared with the AAB "Apple" in this study, or with other bananas in other studies (Stover 1987, Ortiz and Vuylsteke 1998).

The significantly longer flowering to harvest period of the Prata accessions indicates a genetic basis for this trait. However, the duration of this interval is known to be strongly influenced by temperature (Stover 1987) when moisture was not limiting. According to Stover (1987), the period from flowering to harvest varies for AAA clones from about 80 days in the hottest months to 120 days in the coolest months, and requires up to 180 days in some ABB clones in tropical climates. According to Stover (1987), depending on the seasonal variation, flowering to harvest ranged from 75 to115 days for the April to June season and from 100 to145 days for the November to January season for the AAA Grand Nain study in Honduras. Although Stover (1987) noted that light may be a factor, he concluded that most of the difference in time from flowering to harvest was determined by minimum monthly temperatures. In Kisii, flowering of the AAB "Apple" accessions commenced in November 2006, among the earliest accessions (the "Sukari Ndizi"), but was delayed until March 2007 for the late accessions (the Mysore and Prata). Therefore, data for the duration of the interval between flowering and harvest were recorded between November 2006 and August 2007. The minimum temperature was lowest in November at 15.5^{0}C and rose to 16.3^{0}C by February 2007, remaining more or less steady thereafter at 16^{0}C (Figure 7.3). According to Robinson *et al.* (1993), time from flowering to harvest took 118 days in summer and 213 days in winter for the AAA Williams in South Africa. In Cameroon, (Stover 1987), at two sites, the

temperature had a significant influence on the duration of flowering to harvest, i.e., in Nyombe (93-99 days at 21.9 – 31^{0}C), compared with Ekona (117-127 days with 19.7 – 27.8^{0}C); (the cultivar names was not given). The days recorded in this study (130 to 211 days) were close to but higher than those in Stover (1987) (70 to125 days for most tropical areas below 500 m altitude, and 110 to 250 days for most sub-tropical areas). The Kisii experimental site of this study, was a tropical area at a high altitude (1500 m above sea level), and experienced low temperatures that contribute to the long periods observed. The rate of fruit maturing from flowering depends on temperature where moisture was not the limiting factor.

7.5.5. Leaves produced and retained

Although there were no significant variations among the "Muraru" clones in the total number of leaves and leaves retained, it was interesting to note that the "Muraru" produced many leaves (47). For the AAB "Apple" accessions, the mean total number of leaves produced (36) is in agreement with Robinson *et al.* (1993), who obtained an average of 36.8 leaves with AAA Cavendish banana cultivars. A short vegetative cyrcle is enhanced by either fewer total leaves or a faster rate of leaf emergence (Robinson *et al.* 1993). From this study, the "Sukari Ndizi" grouping which had fewer total leaves, was also the earliest to flower and to get ready for harvest. Banana plants that produce more leaves generally take longer to flower. A high correlation did occur between the total number of leaves and the length of the vegetative phase of bananas (the "Muraru" and "Sukari Ndizi" bananas); this agreed with the findings of Ortiz and Vuylsteke (1998). When various banana groups were

evaluated at the Onne station in Nigeria, the mean number of leaves produced was 33, and ranged from 25 to 39 (Ortiz and Vuylsteke 1998), the mean number of days to flowering was 258. Both means are lower than the means obtained in this study. The difference between groupings was an indication that the total number of leaves a banana produces dependent has a genetic basis. However, the rate of leaf production was strongly influenced by temperature. The optimum rate of leaf emergence in Guadeloupe was at 28-30 ^{0}C (Stover 1987). Although the monthly leaf production on average at the beginning of the study was between three and four leaves; there was a general decline in the number of leaves produced as the plant approached flowering. Minimum and maximum temperatures were 15.3^{0}C, and 28.8^{0}C respectively.

Leaves are used in photosynthesis; therefore, at any given time, plants that have more leaves will produce more food, all other factors being constant. From flowering, the banana plants need to make a lot of food for growing fruit, so the plants that have few leaves are probably at a disadvantage, in terms of the fruit filling rate and final bunch weight at harvest. While the mean number of leaves on "Apple" banana plants at flowering was 13, the Prata accessions had more leaves at flowering; this probably gave them an upper hand, resulting in bigger bunch weights at harvest while the "Sukari Ndizi" accessions were more disadvantaged by having fewer leaves at this important stage. For AAB "Apple" bananas, the mean number of leaves at harvest was eight. The Silk grouping remained the one with the least number of leaves at harvest, compared to other groupings, due partly to Fusarium wilt that affected the Silk and the "Sukari Ndizi" accessions to a greater degree than other

groupings. This reduction in the number of leaves by disease may have contributed to the low yields, since leaves are the main photosynthetic part of the plants.

7.5.6. Suckers produced

The number of suckers a banana produce can be a positive or negative trait, depending on the actual number. When too many suckers are produced, they may affect bunch weight because of competition in resources. As a result excessive suckers may need to be removed or destroyed to allow only the few needed for the ratoon crop. Resources such as labor and time would be needed to control too many suckers. On the other hand, when the suckers are too few, the crop to crop cycle from one ratoon to the next may be too long and this also negatively affects production. An ideal plant, therefore, is one that produces just enough suckers. In most plantations suckers must be controlled by destruction. If, however, one is interested in using the suckers to expand an orchard, then greater sucker production is desired. The tetraploid GT and Mysore had good suckers' production of about nine by harvest, whereas the AAB "Apple" bananas in general, had a mean of 13 suckers. Monthly leaf and sucker emergence were some of the phenomena affected by prevailing weather conditions, but they were also highly dependent on the genetic makeup of the plant.

7.5.7. Bunch and fruit characteristics

The mean number of hands per bunch for AAB "Apple" bananas was eight, compared to those of the AAA Cavendish group with a mean of 12.3 hands

(Robinson *et al.* 1993). Because of the significant difference observed among AAB "Apple" banana groupings, this trait gave an indication of the kind of genetic diversity available. The "lax" appearance of Silk bunches is a result of a few hands widely-spaced, while the Mysore had a very compact bunch appearance, because of the very many, closely spaced hands. The Prata and "Sukari Ndizi" accessions were in between with compact bunch appearance.

The number of fruit per hand also contributed to the bunch appearance whether lax, compact, or very compact; many hands give the bunch a more compact appearance. While the mean number of fruit for AAB "Apple" bananas was 13, the AAA Cavendish cultivars had a mean of 22.5 fruit per hand (Stover 1987). The female hands form the bunch, and the number of female hands may be determined at, or even after, the floral initiation stage (Stover 1987). It seemed, therefore, that an upper limit to the fruit number was set at an early stage of inflorescence development, but the actual number achieved may fall short of the limit by an amount determined by the conditions during differentiation; this was, therefore, a trait that can be used to show the genetic diversity within the AAB "Apple" bananas.

As a result of having many fruit per hand and many hands per bunch, the Mysore had the most fruit per bunch. However, the fruits were small in size and weight. For the Silk accessions, the few hands sparely distributed in the bunch resulted in having fewer fruit in the bunch. The Prata accessions also had few, but larger, fruit per bunch.

Some people prefer large fruit while others desire a small fruit that they can consume in one sitting. This trait is complex, in terms of the decision of which

banana type is superior. However, the high fruit weight contributed to the large hand weight of the Prata accessions, makes them generally superior in this category, compared to other AAB "Apple" dessert bananas.

CHAPTER 8

GENERAL DISCUSSION

Morphological and molecular approaches were used to assess the genetic composition and genetic variation of the East African AAB "Sukari Ndizi" and AA "Muraru" dessert bananas. Clearly identifiable combinations of character states allowed recognition and classification of these two distinct groupings. Horticulturally superior varieties among the two dessert banana groupings were also recognized.

8.1. Use of molecular and morphological tools to assess variation and identify AAB "Sukar Ndizi" and AA "Muraru" bananas

Microsatellites, 2C DNA value, and morphology were useful in distinguishing the various bananas groups. The AAB "Apple" dessert banana accessions were distinguished using both cluster and principal component analysis of molecular data. Analysis of morphological characters largely corroborated the microsatellite findings. The microsatellite markers and morphological traits were also able to separate the "Muraru" AA dessert bananas. Both tools showed the AA "Muraru" subgroup of dessert bananas as a homogenous group, with high similarity to commercial AAA Cavendish and Gros Michel dessert bananas.

8.1.1. Determination of the distinctness of the East African AAB "Sukari Ndizi"

Four distinct AAB "Apple" dessert banana taxa were recognized using molecular, cytometric and morphological data. These are Prata, Mysore, Silk and "Sukari Ndizi" (Chapters 3, 4 and 5). The "Sukari Ndizi" is the most homogeneous

of these taxa and is clearly differentiated from the others with the same genomic composition.

The ploidy level of "Sukari Ndizi" and the relationships among the AAB "Apple" bananas was not clear from earlier literature. Shepherd (1957) placed three taxa (Silk, Mysore and Prata/Pome) within the AAB "Apple" dessert banana subgroup of *Musa* AAB group, whereas he grouped Sukari and Wangae accessions (now known to be "Sukari Ndizi" accessions) with AB diploid types such as Safet Velchi, Lady Finger, Kisubi, and Ney Poovan. The assumption was that Sukari (later "Sukari Ndizi") and Kisubi were both AB diploids and closely related or belonging to the same clone. Stover (1987) listed the principal clones of AAB group as: Plantain sub-group, Pisang Kelat, Pisang Raja, Mysore, Maia Maoli, Silk, and Pome. He noted that Pisang Raja (probably closely related to Rajapuri) is not known in East Africa, but Mysore is known as Kikonde in Zanzibar, while Pome is represented by Kijakazi in Zanzibar and Silk is represented by Kipukusa and Sukari in East Africa. Thus Stover (1987) grouped the "Sukari Ndizi" and Silk in one taxon. "Sukari Ndizi" was neither an AB genotype nor a member of the AAB Silk taxon. In this study, "Sukari Ndizi" dessert bananas form a separate distinct cluster within the AAB Apple subgroup, separate from AAB Silk, AAB Mysore, and AAB Prata/Pome, and also AB Safet Velchi taxa. They have close similarity to AAB Prata dessert bananas, and the closest diploid to AAB "Sukari Ndizi" and AAB Prata dessert bananas was AB Kisukari from Zanzibar. I am not aware of the attributes of this diploid banana; however this would probably be a logical choice for use in an improvement program for "Sukari Ndizi". In Zanzibar, however, based on the samples collected, there was

no bona fide "Sukari Ndizi" accession. The other AB accessions, Kisubi and Safet Velchi, are more closely related to the AAB Silk.

In their report, Karamura *et al.* (2006) stated that other bananas, including "Apple" bananas (*Musa* AB/AAB), Bluggoes , Monthan, and *Musa* ABB Pisang Awak, *Musa* AAA Gros Michel and Cavendish are introductions to East Africa during the 1950s. It is possible that AAB "Sukari Ndizi" was introduced with other bananas around this time and has become widely distributed in the region. The only other accessions similar to "Sukari Ndizi" in this study were the reference clones from Bioversity International, ITC 1275 Yangambi no2 and ITC0737 Kingala1, which were probably from the East African region, based on their names. The East African "Sukari Ndizi" was a distinct group that has not accumulated much local variation as shown from the morphological and horticultural traits. This lack of variation indicates that they were, indeed, probably just a recent introductions to the region compared with AAA East African Highland bananas that have diverged into five distinct clone sets (Karamura 1998).

The AAB "Apple" sub-group of dessert bananas is heterogenous, and the difference between taxa within AAB "Apple" sub-group is less than that between the AAB "Apple" and AAB plantain sub-groups. This tends to validate the natural grouping of the AAB "Apple" dessert bananas. Secondly, AAB "Sukari Ndizi" was an homogeneous taxon within the AAB "Apple" sub-group thus justifying it being a distinct taxon separate from the other AAB "Apple" dessert bananas.

8.1.2. Determination of the distinctness of East African AA "Muraru"

The East African AA bananas from Arusha, Tanzania, and Thika and Kisii, Kenya, identified initially by morphological characteristics as belonging to AA "Muraru," formed a homogenous cluster. Based on microsatellite markers, the AA "Muraru" cluster was separate and distant from other AA diploid bananas in the study, an indication that they were a distinct group of AA diploids with a natural grouping separate from the other AA bananas. The ploidy determination, using flow cytometry, also confirmed that "Muraru" were AA diploids. Of much interest was the high similarity between the "Muraru" taxon and the commercial triploid dessert bananas. Accession Kamunyilya, a AA "Muraru," was even in the same microsatellite-defined cluster as Gros Michel, an indication that it was even more closely related to Gros Michel and Cavendish than it was to the other AA "Muraru" accessions. It may be hypothesized that AA "Muraru" bananas are of an origin that is distinct from the other AA in the study sample. The AA Kipaka from Zanzibar, however, proved to be closest to the other AA diploids, such as Sucrier, Maia Hapai, ITC0392 Datil, ITC0714 Kirun, and ITC1358 Ngu. Kipaka was probably of similar origin with these latter accessions. Simmonds and Shepherd (1952) recorded *acuminata* outliers found in Pemba (along the coast of East Africa), and Hawaii and Samoa (*banksii* sub-species). In Pemba, the species was phenotypically not unlike the sub-species *malaccensis*, but, in crossing behavior, seemed nearer to an Indonesian form of the species. Zanzibar is very close to Pemba, and Kipaka is probably the AA outlier mentioned by Simmonds and Shepherd (1952).

In his book, Stover (1987) indicates that the only important edible diploid AA *acuminata* is the Sucrier favored for its sweet, thin-skinned fruit. In the morphological appraisal, AA Sucier has many features similar to AA "Muraru," especially the male bud and flower. These two clones, however, had low similarity and did not seem to be closely related, based on the SSR markers. The Sucrier fruits are also much smaller than those of "Muraru." In this study, AA "Muraru" can be clearly distinguished from other dessert bananas. The Kipaka or Paka, sourced from Zanzibar on the coast of East Africa, was a Sucrier, according to the SSR evaluation. However, the "Muraru" clones from the interior of East Africa were a separate clone group of AA bananas. Simmonds (1966) noted that the *M acuminata* species is extremely variable and the variability is geographically discontinuous, and *M. acuminata* also have morphologically distinct populations that occupy distinct geographical areas and breed mainly with themselves. In the case of the "Muraru" accessions, the AA Mshare has colonized the Meru-Kilimanjaro axis (De Langhe *et al.* 2002) and the AA Muraru–Mucuuru are grown on the Mount Kenya foothills (Karamura *et al.* 2006). Karamura *et al.* (2006) noted that the endemic banana cultivars, considered to have evolved in the East African region include the *Musa* AA Mshare and *Musa* AAA Ilalis of the Kilimanjaro and Usambara ranges, and the *Musa* AAA-EA East African Highland bananas in the great lakes region of East Africa largely found in subsistence systems/small holdings on altitudes between 1000-2000 meters above sea level. The separation of the diploid AA "Muraru" from other diploid *M. acuminata* was an indication that the "Muraru" were indeed distinct diploids AA and that the AA

"Muraru" was a homogenous group, as shown in this study (probably a sign of their natural grouping separate from the other AA bananas).

8.2. Assessment of relative merits of study tools: microsatellites and morphological markers

Comparison of microsatellite markers and morphological markers of phylogenetic relationship showed that they were complementary tools. The relationships in the two phenograms were not exactly the same in all instances, but were similar. The cluster analysis using morphological traits indicated that the AAB dessert banana types were more similar to AAB plantain types, relative to the AAA and AA dessert types. On the other hand, microsatellites showed that the AAB dessert types were relatively more similar to the dessert AAA and AA bananas. The morphological phenogram gave a phenetic depiction of relationships, and the molecular one, a more phylogenetic picture. Molecular data were based on heritable characters, and their description was unambiguous, unlike morphological data that may be ambiguous, e.g., "partially elongated." Molecular traits also evolve in a much more consistent manner than do morphological traits, and avoid the problems of convergence. They can consequently show unambiguous picture of the relationships among organisms (Graur and Li 2000). Separation of cultivars using morphological characters is useful in the field situation. In this study, many morphological characters (84) were used (Chapter 5); and this could explain why there was good correlation between morphological and molecular classifications.

Microsatellite markers are widely and uniformly distributed throughout the nuclear genomes (Ellegren 2000, 2004). This makes them potentially useful in breeding as molecular markers for marker-assisted selection for traits that are of horticultural importance that may be closely linked to certain microsatellite markers. Because banana occupy large land areas when grown and also take a very long time to develop and to select, marker-assisted selection using microsatellite markers (Crouch *et al.* 1997) can avoid this waiting period and also help in the reduction of the number of accessions selected for further field evaluation.

8.3. Proposing names

Any change in cultivar classification will certainly necessitate a change in nomenclature. The ICNCP states that the purpose of giving a name to a distinguishable group of plants is to supply a means of referring to it and to indicate to which category it is assigned. If an assemblage of plants had one or more attributes that make it worth distinguishing, then it may be given a cultivar or Group name (article 18.2 ICNCP). The name of a Group is the correct name of the genus or lower taxonomic unit to which it is assigned together with a Group epithet (article 20.1 ICNCP). On the bases of this study, I propose that the unique East African AAB dessert banana be named AAB "Sukari Ndizi," because the name was the most commonly used to refer to it in the areas where it was found. I also propose that the unique cluster of diploid AA bananas found in East Africa be named AA "Muraru," because this name or those very similar in pronunciation or spellings have been used to refer to them in the areas where they were found. Thus, these names will be easy

to accept by the people familiar with these materials, and, at the same time, will ease communication among scientists.

8.4. Identification of horticulturally superior accessions

This study was also able to identify superior accessions based on horticultural traits. Highly significant differences were found among the various groupings of the AAB "Apple" subgroups in the number of days it takes to flower and to harvest, and also in bunch weight and fruit characteristics. Of great interest to growers was the yield that was calculated based on bunch weight and the number of days taken to harvest (Chapter 7). Two superior groups, namely, Prata and GT accessions, were identified and will be introduced to farmers. These two were also not affected by Panama disease, which attacks the Silk and "Sukari Ndizi" accessions.

Within the "Muraru" taxon, it was not easy to choose desirable clones as all appear to be similar in most characteristics. However, TT2 matured earlier than most of the accessions and had a big bunch weight with long fingers that would make it more attractive to farmers. Muraru Mshale and Muraru accessions also had a big bunch that would make it more preferable. The "Muraru" taxon, however, had two major limitations, i.e., it took too long to mature, and it remained generally green to green-yellow when ripe. However, it was resistant to Panama disease, a trait that makes them more desirable.

8.5. General relationships among the outgroup bananas

Although most of the accessions from the various banana groups, like AAB African and Polynesian plantains, AAA Cavendish, Gros Michel, AAA East African highland banana, AA Sucrier, wild *acuminata*, and *balbisiana*, were originally brought into the study for use as outgroups, interesting results were obtained on the phylogenic relationships between these groups. The African plantains were very closely related to the Polynesian plantains, especially the Maoli and Popoulu accessions, which were closer to the African plantains than Iholena (Chapters 3 and 4) in agreement with the studies of Lebot *et al.* (1993). The AAB plantains were also close to the ABB cooking bananas such as Bluggoe, and Ngoja, but not to ABB Pisang Awak, classified subjectively as dessert (Jenny *et al.* 2003). From the relationships, improvement of the AAA East African highland banana and AAB plantains banana could be accomplished by using the diploid closest to them, namely, Pa Rayong, Higa banksii, Mjenga Michel, and Pisang Masak Ayer.

Although Simmonds (1959) noted that the subjective classification based on "use" does not define any botanically meaningful class, in this study, the molecular classification agrees with the subjective classification. Using PCA, the relative positions of AAA cooking cultivars of East Africa and AAB Plantain were closer and in the same quadrant by microsatellite markers and separated from the dessert cultivars, composed both of AAA and AAB genomes (chapter 4). This was probably an indication that the separation on the basis of "use" has genetic basis, for example, AAA beer bananas have astringent taste and AAB "Sukari Ndizi" have a hard texture

when cooked. Some genetic background that has been captured by microsatellites must be conferring the properties that make them suitable for different uses.

Carreel *et al.* (2002) found that the cultivated bananas like Gros Michel, Cavendish, Silk, Plantain, and East African cooking bananas shared chloroplast pattern with *errans* (35), *banksii* (18) and only two of the *malaccensis* sub-species. Their pattern of grouping showed the cooking (AAB plantains and AAA East African highland, some Saba and ABB) are of type V chloroplast pattern, and that dessert are of type II chloroplast pattern (Jenny *et al.* 2003). This agrees with the data obtained here based on combined nuclei and chloroplast microsatellite markers. This was probably because pollen was normally haploid, while megaspores were haploid, diploid, or triploid, and the maternal genome included the chloroplast. The maternal genome contributed more to the genotype of most triploid cultivated bananas than the paternal genome.

8.6. Suggestions for future research

- A study to generate more chloroplast and mitochondrial microsatellite primer pairs for bananas would set the stage for use of these markers. These when used would then give a better picture of the relationships between the various bananas.
- In this study, the microsatellite allele dosage was not captured. Some alleles were reported to consistently show that the microsatellite marker ma1 27 had similar allele report for Mysore versus Silk, Pratas and "Sukari Ndizi" but the peak heights of alleles 122 and 132 were consistently different between Mysore and the other taxa. Future research should look into ways (e.g., fluorogenic 5′ nuclease (TaqMan)) that can capture the allele dosage when using microsatellite markers with polyploidy bananas. This would make the use of microsatellites markers in banana characterization more powerful.
- Microsatellite markers are widespread in the genome and some maybe closely linked to certain genes of horticultural importance, thus microsatellites have potential to reveal more in bananas. There may be microsatellite markers that could be diagnostic for the various genomic group and banana sub-groups, for example marker ma3-103, which showed allele 150bp to be present only in the AAB and ABB genomes. Future research should sample more microsatellite markers combined with capillary gel electrophoresis for identification of diagnostic markers.

- In the flow cytometry, banana ploidy level was easily determined using any flow cytometer. Research on prediction of the genomic composition based on 2C DNA value should use more replicate run in uniform conditions to achieve better predictive results.
- Horticultural evaluation should extend to 3rd and 4th harvest cycle to be able to capture more information on the potential of banana accessions.

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